Estuaries and Coasts

Comparing the trophic impact of microzooplankton during the spring and autumn blooms in temperate waters

--Manuscript Draft--

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Abstract:	To appreciate coastal trophodynamics, it is necessary to understand the dynamics and control of the spring and late summer/autumn phytoplankton blooms. Classically mesozooplankton are considered as main players in these blooms. Microzooplankton likely also are important in these dynamics, but their role is poorly understood. Critically, due to their rapid generation times, microzooplankton may exhibit rapid shifts during blooms. Through field sampling and rate measurements (dilution experiments) in a well-studied temperate coastal ecosystem (Helgoland, southern North Sea) we ask if there are differences in the trends exhibited between and within the spring and late summer/autumn blooms. To achieve this, we examined early, mid and late bloom periods in both seasons. We found 1) a shift in trophic composition during both blooms, with a trend from strongly autotrophic mixotrophs (e.g. Mesodinium) to mixotrophs and then towards heterotrophs; 2) an increase in intraguild predation at the end of the blooms; and 3) although microzooplankton were major consumers of the spring bloom (grazing coefficient g: 0.23-0.25 d-1; daily percent loss of production Pp: 36-47%), they were unlikely to control it, while in contrast, microzooplankton appeared to play a major role in controlling the late summer/autumn bloom (grazing coefficient g: 0.14-1.53 d-1; daily percent loss of production Pp: 24-103%). In doing so, we suggest that any simplifications that consider these seasonal blooms to be relatively homogeneous and similar will lead to substantial errors in the assessment of coastal trophodynamics.

Dear editors in Estuaries and Coasts,

This manuscript “Comparing the trophic impact of microzooplankton during the spring and autumn blooms in temperate waters” is here submitted for publication in <Estuaries and Coasts>. I am pleased to confirm that the manuscript contains only original data that have not been submitted or published elsewhere. Its submission has been approved by all co-authors, among whom Prof. Karen Wiltshire and Dr. David Montagnes are English native speakers and proficient in the English language. In terms of contribution, experiments and analyses were carried out by J.P. Yang with advisory help of the other authors, and the manuscript was done in close cooperation of all authors. In addition, we understand that the review process will include plagiarism detection, and we guarantee that the manuscript has been prepared according to the ESCO aims and scope and the instructions for authors.

We would be grateful if you could consider the publication of this manuscript.

Yours sincerely,

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Dear Associate Editor Dr. Wim J. Kimmerer and the anonymous reviewers,

We are grateful for your constructive comments on our revised manuscript (No. ESCO-D-19-00345_R1). We have now considered all the suggestions and have revised the manuscript accordingly. Below we provide the comments, followed by our actions.

Comments from the Associate Editor:

1. The responses to comments appear thorough, but I note that one of them claims a change that I can't find: "We have now conducted correlation analysis to support our observations (see Results)." There is a reference (lines 114-115) to conducting a correlation analysis but I could not find results of this analysis.

Author response: We had revised this in the last revision, as the reviewer required. It was missed probably because we did not provide line numbers in the response. We apologize. You can now find the results of correlation analysis at L189-193:

> "The annual cycle of chlorophyll *a* to some extent reflected diatom abundance ($r = 0.64$, $p < 0.01$, Fig. 1b, c) but was apparently also influenced by seasonal dynamics of phytoflagellates ($r = 0.56$, $p < 0.01$, Fig. 1c, e), dinoflagellates ($r = 0.65$, $p < 0.01$, Fig. 1b, c), and red *Mesodinium* spp. ($r = 0.47$, $p < 0.01$, Fig. 1c, d)."

2. The reliance on statistical "significance" to decide whether to include results of grazing experiments is inappropriate. First, please read the important article by Smith in ESCO: https://doi.org/10.1007/s12237-019-00679-y, which follows on numerous such articles elsewhere exhorting authors to drop the use of "significance" as if it meant importance. Consider the null hypothesis implied in a significance test of grazing: H0 = there is no grazing. However, if the grazers are present there must be some grazing, so a priori the null hypothesis is not true. If the null hypothesis is known to be false, the test itself is illogical and invalid and the p-value it produces means nothing. What I think you really mean by saying "NS" is that the combination of natural variability, experimental error, the true amount of grazing, and your experimental design gave a result that you could not distinguish from zero. So instead of indicating "NS", just present the results. However, I strongly recommend replacing the bar plots + SE (which is uninterpretable without the degrees of freedom) with boxplots and dot-plots that give more information about the results.

Author response: We fully accept your suggestion to adopt arguments made by Smith and others to remove "significant" from our reporting of results. To this end, in the text (L167-168) we have now made it clear that in the experiments where we could not reject the null hypothesis, this means we have not been able to detect grazing (at $\alpha = 0.05$), and we have provided the p and df values for these tests (on the figure). Please note that for our (very standard, e.g., Landry and Hassett 1982; Calbet et al. 2008; Calbet et al. 2012) dilution experiments, regression is applied, e.g., grazing is determined as the slope of the regression of phytoplankton growth rate vs dilution. In this case, the null hypothesis is (as you indicate for reasons that you outline above) that no grazing was *detected*. In these experiments, through regression, researchers obtain estimates of the coefficients *g* and *k* (with associated SE values). The SE of these parameter values can, of course, then be converted to a 95% CI by multiplying it by a critical *t* value determined by df. However, the SE is commonly used in

the reporting of these experiments in the literatures and we would like to be consistent with these. In addition, Reviewer 2 has already asked us to provide SE. Therefore, we have retained this error estimate in our data presentation. However, for such data it is impossible to provide the results graphically as box and whisker plots. The reason for this is that the parameters and their error terms are obtained from a single regression (in our rigorous experiments with replicate measurements of the response at each dilution level). As is virtually always the case for such dilution experiments (since their development, Landry and Hassett 1982), true independent replication of the experiments is not performed. This then precludes presenting data as box and whisker plots. We have, therefore, again followed standard practice and provided parameter estimates with their SE as error bars.

Landry MR, Hassett RP 1982. Estimating the grazing impact of marine microzooplankton. Marine Biology 67: 283-288.

Calbet A. et al. 2008. Impact of micro- and nanograzers on phytoplankton assessed by standard and size-fractionated dilution grazing experiments. Aquatic Microbial Ecology 50: 145-156.

Calbet A. et al. 2012. Effects of light availability on mixotrophy and microzooplankton grazing in an oligotrophic plankton food web: Evidences from a mesocosm study in Eastern Mediterranean waters. Journal of Experimental Marine Biology and Ecology 424-425: 66-77.

3. Too little information is given about several aspects of the paper. Specific examples are provided below, but the results cannot be interpreted without more information about the methods, including those used to get the data gathered by others. This could be in a supplement, but it needs to be available.

Author response: As requested, we have now added the essential information in the Electronic Supplementary Material:

"Surface water samples were taken from the Helgoland Roads long-term station "Kabeltonne" on a daily to weekly basis (see Appendix 1 for details of dates).

For ciliates, a 250 ml sample was fixed with acid Lugol's solution (2% final concentration) and stored at 4 °C in the dark. Then, a 50 ml subsample was settled in a Utermöhl chamber for at least 24 h. The whole chamber was examined at 200-fold magnification under an inverted microscope (Zeiss Axiovert 135). Following this procedure, ~20 individuals of the most abundant taxa were observed. Some ciliates were only identified to genus level or placed into size groups or morphotypes, as Lugol’s obscures some diagnostic characters.

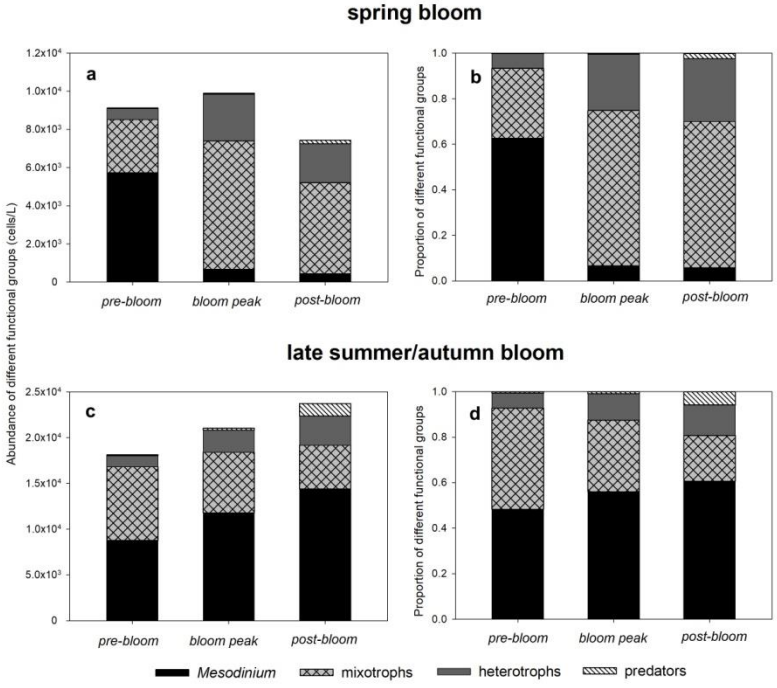
The data for environmental variables (DIN, phosphate, silicate, and Chlorophyll *a*), phytoplankton, and dinoflagellates were obtained from the routine monitoring at the "Kabeltonne" site, where biological, chemical and physical parameters are recorded continuously on a work-daily basis (Franke et al. 2004). Following similar methods as described for ciliates above, the phytoplankton and dinoflagellate species numbers were counted to species level wherever possible. Only those data corresponding to the date of collection for ciliate data were used in our analysis (Appendix 1).

Mesozooplankton determined to species level have been included into the routine monitoring at the "Kabeltonne" site since 1975 (Greve et al. 2004). Samples were collected

using a HYDROBIOS quantitative collection hand-net (mesh size 150 µm, net length 100 cm) on a daily to weekly basis. Copepod data used in our analysis (Appendix 1) were taken from the mesozooplankton dataset and the taxa included *Acartia* spp., *Alteutha interrupta*, *Amphiascus* sp., *Calanus* spp., *Centropages* sp., *Corycaeus anglicus*, Cyclopoidea, *Ectinosoma* sp., *Euterpina acutifrons*, *Harpacticoida* sp., *Microcalanus* sp., *Microsetella* sp., *Monstrilla helgolandica*, *Monstrilla* sp., *Oithona* sp., *Oncaea similis*, *Parathalestris* sp., *Pseudo-/Paracalanus* spp., *Temora longicornis*, *Thalestris longimana*, *Tisbe* sp., and nauplii."

4. Figure 2 is inadequate for supporting claims about changes between and within bloom periods. It displays proportions of trophic groups from unknown numbers of samples, in unstated units (biomass, cells?). It fails to cast the calculated differences in the context of variability, and thereby cannot support any claim about these apparent changes. I am not arguing the changes are not real, just that this graph does not support the statements made. Also since these are relative amounts, it is unclear whether (e.g.) predators increased in absolute abundance or everything else decreased.

Author response: This was an excellent suggestion. We agree that it is important to show the abundances of different functional groups together with their proportion. Therefore, two plots (Fig. 2a, c) have been added to show the absolute abundance of each functional group during the blooms.



5. The paper argues that grazing by the abundant microzooplankton may be the “cause” of the decline in the fall bloom (in contrast to the spring bloom). However, the decline in the autumn bloom seems well explained by the decline in light levels. Longhurst, A. 1995. Seasonal cycles of pelagic production and consumption. Prog. Oceanogr. 36, 77–167, doi:10.1016/0079‐6611(95)00015‐1. Obviously both growth and mortality must figure into any assessment of causes of changing biomass, but the paper gives no indication that this is the case, or how the magnitudes of growth and mortality of phytoplankton change (necessary to determine the role of mortality in the decline).

Author response: As the light cycle (see Table 1) and intensity were quite similar during both periods of our grazing experiments, but grazing rates were higher in the autumn, we suggest that light may be not the only factor at this point leading to the decline in phytoplankton. However, we agree that light will influence the phytoplankton growth, and should also be taken into consideration when conducting experiments in case of large light variation. To indicate this, we have now added appropriate recognition into the Discussion (L367-371).

6. I suggest switching Figures 1 and S1. Figure S1 is more relevant to the study, being the data gathered when the experiments were done. It also indicates when the experiments were run to allow a comparison with the field data. Figure 1 apparently presents data aggregated (by methods not fully explained) among several years, and is therefore useful only for showing how the study year compared with what could be called typical patterns.

Author response: We would prefer to keep Fig. 1 in the introduction of the manuscript because it provides important context for the study based on 4.5 year data (the collection of which is now explained as requested). An underlying assumption of this study is that the waters around Helgoland are characteristic of temperate coastal ecosystems. Fig. 1 serves as a synthesis of existing data, providing the background to support this assumption. In this context, the annual patterns achieved from 4.5 years are more convincing than those from only one year. In contrast, Fig. S1 is provided for methodological purposes, to illustrate that the year when experiments were conducted was typical of annual patterns (Fig. 1). We emphasize that this modification (to move Fig. S1 to the supplementary material) was made according to the advice from Reviewer 2 (please refer to Point 15 of specific comments) in the last round. We agree with this Reviewer, especially given the modern accessibility of supplemental material.

Specific comments:

1. 60 & 236: While I think the Miller & Wheeler textbook is a good one, perhaps the authors could cite instead some of the peer-reviewed primary or review literature on this topic, for example:
Sverdrup, H. U. 1953. On conditions for the vernal blooming of phytoplankton. ICES J. Mar. Sci., 18, 287–295.

Author response: We agree with you that it is appropriate to provide credit where it is due and in doing so use the original, primary literature. We have, therefore, taken your advice and cited the classic work by Sverdrup (1953). We also feel that it is useful to indicate the continued, general acceptance and elaboration of such "facts" and, critically, to indicate where they can be found in easily acceptable, recent sources. Finally, it seems appropriate to suggest that these are "text-book" phenomena by citing a text-book, albeit an advanced one. We, therefore, have also retained the reference to Miller and Wheeler.

2. 109-120 Very sketchy information provided. For example, how were these samples collected? What was the protocol for preservation and counting? In the case of the mesozooplankton, what mesh net was used and what organisms were included in the count (e.g, nauplii)?

Author response: We have revised this section, as mentioned above (refer to Point 3 above).

3. 113 “the same site” unclear.

Author response: We have revised this, as you can see in L113.

4. 117-118 Running averages (is that means, medians, or what?) for what purpose? It seems better to present the raw data than to smooth out the wiggles.

Author response: Here we have now clarified that the “average” was a mean (L118). Using this running average was a decision that we did not make lightly. We first plotted all the data. Because of considerable variation (natural, as methods have been consistent) in the data, trends were obscured. We contemplated and explored fitting mechanistic trends to the data, but as this was not a key part of the study, we decided on the simpler, and for our purposes equally useful, approach of applying a running average. As is clear, this allowed us to make our case. However, we agree that the raw data may be useful to others in the future. To this end, we have provided the raw data as an Appendix in the supplementary materials.

5. 127-128 gives the volume settled, which by itself is uninformative – how much of the chamber was examined, how were samples examined, how many cells counted in a sample, what taxonomic resolution?

Author response: We have added this information in L128-135:

> "Microzooplankton abundance and taxonomic composition were evaluated by settling 50 ml samples in Utermöhl sedimentation chambers for at least 24 h and examining the whole chamber at 200-times magnification under an inverted microscope (Zeiss Axiovert 135). A minimum of 20 individuals of the main taxa was counted by this method. Due to difficulties that can occur with the identification of microzooplankton after fixation with acid Lugol's solution, some dinoflagellates and ciliates were only identified to genus level or placed into different size groups based on their similar shape (e.g. for some taxa in the complex genus *Strombidium* and scuticociliates)."

6. 143 “To tested..”

Author response: We have revised this, L151.

7. 166-167 “this…. rates” something missing here

Author response: We have revised this, L177.

8. 193-194 Inaccurate: Chlorophyll had already increased from ~ 1 to ~2 ug/L by the time Mesodinium spp. became abundant (at least on the graph).

Author response: Red *Mesodinium* spp. are known to be a substantial part of chlorophyll biomass (Please refer to our response to the comment 2 from the Reviewer 2). Considering the low abundance of diatoms, the increase of chlorophyll would mainly result from high number of mixotophic, red *Mesodinium* spp., and therefore, it was still at the pre-bloom stage of the diatom bloom in spring.

9. 194-196 Mixotrophs: where is this shown? In Figure 2 I suppose, but there is no way to assess the reliability of the statements here from that figure; it is too aggregated.

Author response: We have added values for the proportions of different functional groups of microzooplankton (L202-212). Along with the newly added Fig. 2a, c, it should now be clear for reader to follow our statements on the compositions:

"During the spring bloom, red *Mesodinium* spp. were abundant in the pre-bloom stage (63%), while mixotrophs dominated the community during the bloom peak and post-bloom stages (68% and 64% respectively) (Fig. 2a, b). The proportions of heterotrophs increased during the bloom peak (25%) and the post-bloom stage (28%) compared to the pre-bloom stage (6%), while the proportions of predators increased during the post-bloom stage (Fig. 2a, b). During the late summer/autumn bloom, red *Mesodinium* spp. accounted for a large proportion at all three stages (48%, 56%, and 61%, respectively) (Fig. 2c, d). Mixotrophs were highest in the pre-bloom period (45%) and then decreased as the bloom progressed (32% and 20%, respectively), while both heterotrophs and predators increased during the process of the bloom (Fig. 2c, d)."

10. 200 "the" missing

Author response: We have revised this, L211.

11. 205 and elsewhere: what does the ± denote? Please say what they are, but if these are standard errors they are uninformative and should be replaced with confidence intervals.

Author response: As indicated above, in the previous revision Reviewer 2 requested that we include SE. Furthermore, the 95% CI are just SE * a critical *t* value determined by df, so we do not feel that they are substantially more informative, especially as we have now added the p values and the df to figures. We have, therefore, now made it clear in the text that the ± denotes SE (L217, L230).

12. 212-213 Suggesting either that it was not pronounced or that variability was high in relation to the actual value and the degree of replication. The microzooplankton probably grew.

Author response: We have revised this text in accordance to this comment and those above, L223-226.

13. 237 See Sverdrup reference above for an explanation of "improved" conditions.

Author response: Following above arguments, here we now have referred to both Sverdrup's original paper and the book by Miller and Wheeler, L249-251.

14. 267 "Clear progression" is not clear to me, if it is based on Fig. 2.

Author response: We have revised the text, L280. Please also refer to our response to the point 9 above.

15. 269 How do you know conditions are "optimal"?

Author response: You are correct. We do not know if the conditions are optimal. We have revised the text acordingly (L282-286):

"This might be expected, as initially light and nutrient conditions will likely benefit taxa such as red *Mesodinium* spp. which tend to rely more on phototrophy compared to phagotrophy according to their relatively low ingestion rates and long survival time

without prey (Stoecker et al. 2017), while due to the lack of food, heterotrophs will be restricted.

16. 277-278 This seems circular: mixotrophs dominate in systems that favor mixotrophs
Author response: We have clarified this, L293.

17. 289 "end of the bloom." Which bloom? The copepods increased after the spring bloom and decreased after the autumn bloom.
Author response: We have clarified that we mean the spring bloom, L305.

18. 291 delete "a"
Author response: We have revised this, L307.

19. 294 "Our most remarkable finding…" That may be so, but if you think it is remarkable why not also make it clear to readers how you know this? The bar graphs don't do it.
Author response: We think that now it is quite clear to see the increase of predators at the end of blooms with the help of addition of Fig. 2a, c. Here, we have now referred to this figure, L311.

20. 295-297 This sentence is difficult to follow.
Author response: We have rewritten this sentence, L311-314:

21. 299-300 This sentence also needs to be rearranged. Who or what exhibited similar trends?
Author response: We have revised the sentence, L315-316.

22. 305 What increase?
Author response: We meant the increase of *Mesodinium* spp. in the community during the late summer/autumn bloom. We have revised this section, L325.

23. 333 Nutrients hardly seem depleted. In Fig S1 DIN hovers around 8 µm, which hardly seems like depletion. Silicate gets down to about 1 µm but at the time of the most rapid decline in diatom abundance it is around 8 µm. I would assume these are good oceanographic measurements (though the Methods could inform about that) and therefore highly reliable.
Author response: We have revised this, L354.

24. 338 and elsewhere use consistent terminology for the bloom.
Author response: We have revised the terminology for the bloom throughout the MS.

25. 347-349 "aid substantially" Since you have growth and grazing rates, perhaps you could estimate the magnitude of the aid at least semi-quantitatively.
Author response: The parameters P_i (percentage loss of phytoplankton standing crop per day) and P_p (percent loss of phytoplankton production per day), calculated from k (growth rates) and g (grazing rates) and shown in Fig. 3c, d, h, i, are two important parameters that

quantitatively estimate the magnitude of grazing impact by microzooplankton. We have now referred to this figure here (L375).

26. 350 A Conclusion section seems superfluous for an otherwise concise experimental paper.
Author response: We agree and have removed the Conclusion section.

Comments from Reviewer #2:

1. The term "long-term trends" is not being used correctly and will cause some confusion, especially with the Estuaries and Coasts readers. Based on the term "long-term trends", I was expecting analysis of how all these factors were changing over the 4.5-year period. However, it appears what the authors actually did was average 4.5 years of long-term monitoring data to look at the typical annual cycle.
Author response: We agree with the Reviewer. We have changed “long-term trends to "annual patterns" (L95, 108, 185, 511) or "annual variation" (L109).

2. The authors refer to Mesodinium as a "functional autotroph" and keep it distinct from the "mixotroph" group. This is not an appropriate term for Mesodinium, they are an obligate mixotroph (Hansen et al. 2013; Stoecker et al. 2017), and need both phototrophy and phagotrophy. While Mesodinium can maintain the chloroplasts that it steals to come degree, they do have to be regularly replaced (about every few days) by consuming more prey. Even more important, Mesodinium is a specialist, which means they only steal the chloroplasts of a few select prey, and they are typically associated with Teleaulax. Saying that the microzooplankton community moves from autotrophy to more heterotrophy over the spring bloom could still stand, but the discussion has to be more nuanced. Mesodinium are a mixotroph that do not produce their own chloroplasts, but when they steal chloroplasts, they can operate as a phototroph for some time. Then the other mixotrophs that take over after Mesodinium, I assume, lean more on phagotrophy compared to phototrophy.
Author response: We agree with the reviewer that *Mesodinium rubrum* and *M. major* are obligate mixotrophs. We took them as a single group mainly as they made up a substantial part in the microzooplankton community. In addition, we assume that they lean more on phototrophy compared to phagotrophy due to the low ingestion rates (equivalent to 1% of the C required for growth) and long survival (up to four months) without prey as explained in Stoecker et al. (2017, Mixotrophy in the marine plankton). Consequently, according to the Reviewer's suggestion, we have deleted the term "functional autotrophs" (L138, 192, 203, 272, 283), and added discussion about *Mesodinium* spp. (L283-285 and L318-322).

3. In a few instances, I wish the authors had presented some more data, but most importantly I think the paper would benefit from presenting the more detailed microzooplankton identification data from the lugol's samples. So rarely when dilution experiments are being done do people actually record what the community composition of the microzooplankton is; this is interesting data. The groups, "mixotrophs", "heterotrophs", and "predators" are vague and I wanted to know what species, genera, etc were associated with these groups. I know Figure 4 presents data for three specific species, but a list of what identified microzooplankton were put into each group and maybe some data on their average abundance in spring and autumn would be very nice.

Author response: We have added Table S1 in the Electronic Supplementary Material to show the species list of microzooplankton with average abundances in spring and autumn, respectively.

4. Line 330-332: This is not an appropriate characterization of Irogoien' loophole hypothesis. This 2005 paper focuses on the start of a bloom, not the decay of the bloom. I did not find a mention of the role grazing plays in the decay of a bloom in Irogoien et al. 2005.

Author response: We have changed the reference to Löder et al. (2011), L353. They reported an average consumption of 120% of the phytoplankton production when mesozooplankton grazers were excluded during the spring bloom.

Comparing the trophic impact of microzooplankton during the spring and autumn blooms in temperate waters

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Abstract

To appreciate coastal trophodynamics, it is necessary to understand the dynamics and control of the spring and late summer/autumn phytoplankton blooms. Classically mesozooplankton are considered as main players in these blooms. Microzooplankton likely also are important in these dynamics, but their role is poorly understood. Critically, due to their rapid generation times, microzooplankton may exhibit rapid shifts during blooms. Through field sampling and rate measurements (dilution experiments) in a well-studied temperate coastal ecosystem (Helgoland, southern North Sea) we ask if there are differences in the trends exhibited between and within the spring and late summer/autumn blooms. To achieve this, we examined early, mid and late bloom periods in both seasons. We found 1) a shift in trophic composition during both blooms, with a trend from strongly autotrophic mixotrophs (e.g. *Mesodinium*) to mixotrophs and then towards heterotrophs; 2) an increase in intraguild predation at the end of the blooms; and 3) although microzooplankton were major consumers of the spring bloom (grazing coefficient g: 0.23-0.25 d^{-1}; daily percent loss of production P_p: 36-47%), they were unlikely to control it, while in contrast, microzooplankton appeared to play a major role in controlling the late summer/autumn bloom (grazing coefficient g: 0.14-1.53 d^{-1}; daily percent loss of production P_p: 24-103%). In doing so, we suggest that any simplifications that consider these seasonal blooms to be relatively homogeneous and similar will lead to substantial errors in the assessment of coastal trophodynamics.

Key words: algal bloom, coastal ecosystem, dilution experiments, grazing, trophodynamics

Introduction

Although coastal waters represent less than 10% of the global ocean surface, they are responsible for about 20% of the oceanic net primary production and globally account for 80% of organic matter burial; they are thus the setting of processes critically important to global productivity and global carbon dynamics (Field et al. 1998). In temperate latitudes these waters show a seasonality with typically two phytoplankton blooms (spring and late summer/autumn) that are classically recognized to be dominated by diatoms but also include phytoflagellates. These blooms are particularly important in the functioning of marine ecosystems in coastal regions as their high primary production can be transferred up the food chain by algivorous grazers (Sverdrup 1953; Miller and Wheeler 2012). An appreciation of coastal trophodynamics is incomplete without a full understanding of the controls on both the spring and late summer/autumn phytoplankton blooms.

Microzooplankton are recognized as important grazers in marine ecosystems (Schmoker et al. 2013), consuming both diatoms and phytoflagellates and up to 60-75% of the daily phytoplankton production (Landry and Calbet 2004). Critically, in contrast to mesozooplankton, microzooplankton respond rapidly to increasing phytoplankton availability (Montagnes and Lessard 1999; Löder et al. 2011) and have long been suggested to be the first exploiters of seasonal blooms (Smetacek 1981; Banse 1982). It has been argued that phytoplankton blooms only occur when microalgae display a positive net growth rate over microzooplankton grazing pressure (Irigoien et al. 2005), suggesting microzooplankton grazing controls the formation and duration of phytoplankton blooms (Schmoker et al. 2013). However, it is now appreciated that "microzooplankton" do not constitute a single trophic group: dinoflagellates can be as important as ciliates; both dinoflagellates and ciliates can be mixotrophic; and predation occurs within the microzooplankton, with ciliates and dinoflagellates ingesting other microzooplankton (e.g. Jeong et al. 2010; Johnson 2011;

Franze and Modigh 2013; Mitra et al. 2014). The microzooplankton community is, therefore, complex, and, although poorly evaluated, evidence indicates that the relative role of these groups may change during seasonal blooms (Montagnes et al. 1988; Löder et al. 2011).

Some field and experimental studies exist on the role of microzooplankton during the development of spring blooms (Smetacek 1981; Fileman and Leakey 2005; Löder et al. 2011). However, the extent to which microzooplankton may structure the phytoplankton communities and their trophic succession during the spring and late summer/autumn blooms are poorly understood, and to our knowledge no study has contrasted their dynamics during both bloom seasons in a single system. Here, by focusing on a sampling program on the two bloom periods, we address these issues in a well-studied temperate ecosystem: i.e., waters around the island of Helgoland (southern North Sea), where spring and late summer/autumn phytoplankton blooms occur as outlined in Wiltshire et al. (2015). We have also taken advantage of the long-term Helgoland Roads dataset that provides comprehensive physical, chemical, and biological data at a daily resolution (Wiltshire and Dürselen 2004), offering us an opportunity to assess the role of microzooplankton and their associated community within and beyond the blooms.

Our aim was to use this system to provide a basis for understanding the role of microzooplankton in structuring the plankton communities and the functioning of the marine ecosystems during the two main seasonal blooms in temperate coastal waters. To do so, we first examined the annual patterns in nutrients and plankton dynamics over several years by analyzing the time-series data, to reveal that the waters around Helgoland are characteristic of temperate coastal ecosystems. We then asked the question: are there differences in the patterns exhibited in the spring and late summer/autumn blooms? Specifically, we examined which functional group dominate the microzooplankton community and evaluated their succession during the development of blooms. We then coupled these observational data with

growth and grazing rate measurements at discrete time points in the bloom period to determine the impact of microzooplankton on the outbreak, persistence, and decline of phytoplankton blooms. This combination of meta-analysis, field sampling, and field experiments provides clear indication that the two blooms differ in terms of the microzooplankton composition, the grazing impacts of microzooplankton and the role of microzooplankton in structuring the plankton communities.

Materials and methods

Identifying annual patterns at Helgoland

Average, annual variation in ciliate abundance was determined from existing data, covering a period of 4.5 years between 2007 and 2012 from the "Kabeltonne" site at Helgoland Roads (54°11.30′N 7°54.00′E), located in the German Bight (southern North Sea). Nutrients, chlorophyll *a*, phytoplankton (diatoms and phytoflagellates), and dinoflagellate data were obtained from a long-term dataset from the "Kabeltonne" site as described in Franke et al. (2004). Correlation analyses between chlorophyll *a*, and the phytoplankton groups were conducted with the SPSS software (Version 19, SPSS Inc.). Copepod data were determined from a wider set of mesozooplankton data (Greve et al. 2004). The data were collected generally on a daily to weekly basis (see Electronic Supplementary Material for detailed information) and are presented as running averages (means, obtained across eight consecutive collection times). Our sampling times for experiments were chosen to reflect patterns during the early, mid, and late diatom blooms in the spring and late summer/autumn (Table 1; Fig. S1).

Determining the plankton composition during the blooms

Water from grazing experiments (see below, collected during the spring and late summer/autumn diatom blooms, Fig. S1) was fixed with Lugol's iodine (2% final

concentration) for phytoplankton and microzooplankton analysis. The abundance and taxonomic composition of phytoplankton were determined according to methods applied in the Helgoland Roads long-term phytoplankton data archive (Wiltshire and Dürselen 2004). Microzooplankton abundance and taxonomic composition were evaluated by settling 50 ml samples in Utermöhl sedimentation chambers for at least 24 h and examining the whole chamber at 200-fold magnification under an inverted microscope (Zeiss Axiovert 135); through this at least 20 individuals of the dominant taxa and fewer of rare species were enumerated. Due to difficulties that can occur with the identification of microzooplankton after fixation with acid Lugol's solution, some dinoflagellates and ciliates were only identified to genus level or placed into different size groups based on their similar shape (e.g. for some taxa in the complex genus *Strombidium* and scuticociliates). Dinoflagellate identification mainly followed Kraberg et al. (2010). Ciliate identification followed Montagnes and Lynn (1991), Carey (1992), and Song et al. (2009). Based on trophic status, microzooplankton were divided into four groups: red *Mesodinium* spp. (*M. rubrum* and *M. major*), which are strongly autotrophic mixotrophs; other mixotrophs; heterotrophs; and predators (based on Jeong et al. 2010; Johnson 2011).

Microzooplankton grazing experiments

Dilution experiments (Landry and Hassett 1982) were conducted during the spring and late summer/autumn diatom-blooms (see Fig. S1) in 2012. Surface (2 m) water was taken from the same location as the long-term dataset (see details above). For each experiment, 70 L was collected, and 40 L was filtered through a 200 µm mesh to remove mesozooplankton. The remaining 30 L was filtered through a 0.45 + 0.2 µm sterile inline membrane filter capsule (Sartobran® 300), providing particle-free water for dilutions. Then, 11 L containers were prepared for dilutions of 1:0, 3:1, 1:1, 1:3, and 1:9 (200 µm pre-screened : particle-free water).

149 To prevent nutrient limitation biases containers were enriched with F/2 reduced by half (40
150 µM nitrate, 0.65 µM phosphate and 1.2 µM silicate, final concentrations) following Löder et
151 al. (2011). To test the effect of adding nutrients on phytoplankton growth, we compared the
152 net growth rates in nutrient amended and non-amended treatments at ambient phytoplankton
153 concentrations (i.e. the undiluted treatments); no significant difference was detected (two
154 tailed t-test, $\alpha = 0.05$), indicating that phytoplankton were not nutrient-limited during
155 incubation.

156 To determine grazing, replicate (n = 4 per dilution) 2.3 L polycarbonate bottles were
157 filled with the water from the 11 L containers using methods that prevent ciliate losses (Löder
158 et al. 2010). Bottles were incubated for 24 h on a rotating plankton wheel at the in-situ
159 temperature and light cycle (Table 1), with similar light intensities for both blooms, according
160 to the Helgoland Roads long-term data. To obtain taxa abundances and composition, initial
161 and final samples from each bottle were processed as indicated above. Growth rates of
162 phytoplankton and grazing rates of microzooplankton were determined following the
163 established methodology of Landry and Hassett (1982), using the equation $Ct_{24} = Ct_0 \times e^{(k-g) \times}$
164 $^{\Delta t}$, where Ct_0 is the phytoplankton concentration at the beginning of incubation, Ct_{24} is the
165 concentration after a one day incubation, k (d^{-1}) is the phytoplankton growth coefficient, g (d^{-1})
166 is the microzooplankton grazing coefficient, and Δt is the incubation time in days. The p and
167 df values are provided for these tests, and in the experiments where the null hypothesis could
168 not be rejected (at $\alpha = 0.05$), this indicates that no grazing was detected.

169 The percentage loss of phytoplankton standing crop per day (P_i) and the percent loss
170 of phytoplankton production per day (P_p) were determined according to Quinlan et al. (2009),
171 based on the values of instantaneous growth (k) and grazing rates (g). Microzooplankton
172 growth rates (μ, d^{-1}) were calculated for ciliates and dinoflagellates respectively, in undiluted
173 treatments assuming exponential growth, $\mu = \ln(N_t/(N_0)/t$, where N_0 is abundance at the

beginning of incubation, N_t is the abundance after one day, and t is the incubation time in days. The method of Landry and Hassett (1982) assumes a non-saturating linear functional response, although later work has indicated that if a saturating response occurs this will be biased predicted grazing rates (e.g. Gallegos 1989; Dolan et al. 2000; Calbet et al. 2008, 2012; Latasa 2014); here we assumed a linear non-saturating response, as the chlorophyll levels during the investigations (0.91-6.15 μg L^{-1}, Table 1) suggested that saturated feeding responses would not be expected as in eutrophic waters (Worden and Binder 2003). To assess the growth responses of microzooplankton during both blooms, one-sample *t*-tests were conducted to determine if the growth rates significantly differed from zero ($\alpha = 0.05$), and they were reported only if significant.

Results

Identifying annual patterns at Helgoland

Nutrients followed a typical annual cycle, with elevated levels in the winter and early spring and reduced levels between the late spring and late summer (Fig. 1a). Diatoms exhibited a classic pattern, with low levels in the winter, fluctuating levels during the summer, and blooms in the spring and late summer/autumn (Fig. 1b). The annual cycle of chlorophyll *a* to some extent reflected diatom abundance ($r = 0.64$, $p < 0.01$, Fig. 1b, c) but was apparently also influenced by seasonal dynamics of phytoflagellates ($r = 0.56$, $p < 0.01$, Fig. 1c, e), dinoflagellates ($r = 0.65$, $p < 0.01$, Fig. 1b, c), and red *Mesodinium* spp. ($r = 0.47$, $p < 0.01$, Fig. 1c, d).

Copepods were rare in the winter, increased in the spring, reached highest abundances during the summer (with fluctuations), and decreased in the autumn (Fig. 1 c); notably, copepods were relatively low during the spring and late summer/autumn diatom blooms (Fig. 1b, c). In contrast, microzooplankton (i.e. dinoflagellates and ciliates, Fig. 1b, e) remained at

high abundances between the early spring and late autumn, with exceptionally high numbers of dinoflagellates in the autumn.

Microzooplankton trophic composition during blooms

There was a shift in trophic composition during both the spring and late summer/autumn blooms, with a trend from autotrophic towards heterotrophic processes (Fig. 2). During the spring bloom, red *Mesodinium* spp. were abundant in the pre-bloom stage (63%), while mixotrophs dominated the community during the bloom peak and post-bloom stages (68% and 64% respectively) (Fig. 2a, b). The proportions of heterotrophs increased during the bloom peak (25%) and the post-bloom stage (28%) compared to the pre-bloom stage (6%), while the proportions of predators increased during the post-bloom stage (Fig. 2a, b). During the late summer/autumn bloom, red *Mesodinium* spp. accounted for a large proportion at all three stages (48%, 56%, and 61%, respectively) (Fig. 2c, d). Mixotrophs were highest in the pre-bloom period (45%) and then decreased as the bloom progressed (32% and 20% respectively), while both heterotrophs and predators increased during the process of the bloom (Fig. 2c, d).

Growth and grazing during the blooms

During the spring bloom, microzooplankton exhibited similar grazing coefficients (g, d^{-1}) on phytoplankton in all three stages with significant grazing on phytoflagellates at the pre-bloom, and on diatoms at the bloom peak and post bloom (Fig. 3a). The phytoplankton growth coefficient (k, d^{-1} ± SE) ranged between 0.62 ± 0.05 and 0.86 ± 0.06 d^{-1} (Fig. 3b). The daily percent losses of phytoplankton standing crop (P_i) due to microzooplankton grazing were similar during the three stages of the bloom, ranging between 26% and 28% (Fig. 3c). The daily percent losses of production (P_p) at the pre-bloom and bloom peak were similar (43%, 47%, respectively), and slightly higher than that of the post bloom (36%) (Fig. 3d). Ciliates

exhibited significant growth with a mean of 0.32 ± 0.06 (SE) d^{-1} at the pre-bloom, while dinoflagellates exhibited a growth rate of 0.21 ± 0.01 (SE) d^{-1} at the bloom peak. None of the microzooplankton exhibited growth rates that were distinguishable from zero during the post-bloom (Fig. 3e). Note that these results do not mean that growth did not occur, just that we were not able to detect it.

During the late summer/autumn bloom, microzooplankton exhibited significant grazing on phytoflagellates at the bloom peak and post-bloom. Grazing on diatoms occurred at all three stages, and the grazing coefficient at the post-bloom was higher than those at the pre-bloom and bloom peak (Fig. 3f). The phytoplankton growth coefficient (k, d^{-1} ± SE) ranged between 1.10 ± 0.10 and 1.86 ± 0.11 d^{-1} for phytoflagellate and between 0.80 ± 0.04 and 1.44 ± 0.06 for diatoms (Fig. 3g). The daily percent loss of standing crop (P_i) on phytoflagellates was 117% and 61% at the bloom peak and the post-bloom, respectively, while for diatoms it was higher at the post-bloom (360%) than that at the pre-bloom (29%) and bloom peak (15%) (Fig. 3h). The daily percent losses of production (P_p) exhibited similar trends to those of P_i with the highest value (103%) occurring at the post-bloom (Fig. 3i). Ciliates exhibited significant growth at all three stages with mean growth rates ranging between 0.73 ± 0.01 (SE) and 0.94 ± 0.03 (SE) d^{-1}, while dinoflagellates exhibited significant growth only at the bloom peak and post-bloom, and the mean growth rates were lower than those of ciliates (Fig. 3j).

Discussion

Embedding microzooplankton in the bloom dynamics of temperate coastal ecosystems

We first demonstrate that the ecosystem around Helgoland in the North Sea exhibits typical patterns of phytoplankton and copepods for spring and late summer/autumn blooms. The annual patterns of nutrients, phytoplankton, microzooplankton and copepods in 2012 (Fig. S1)

246 when we conducted the grazing experiments were similar to those based on the averages of
247 4.5 year data (Fig. 1), indicating the results are from a typical year and its usefulness for the
248 assessment of microzooplankton dynamics during such blooms. It is well established how
249 such blooms occur (Sverdrup 1953; Miller and Wheeler 2012): the rapid growth of diatoms
250 and phytoflagellates in the spring is due to improved light and temperature conditions, in
251 combination with high winter-nutrient levels. During blooms, copepod numbers are low, but
252 they increase in the summer and decline in the autumn. Likewise, the late summer/autumn
253 bloom of autotrophs is caused by mixing and influx of sub-thermocline nutrients, while light
254 levels are still high. What is less often reported, but is also well established, is that during
255 blooms, when copepod abundance is low and autotrophs are abundant, microzooplankton
256 abundance is high (Smetacek 1981). Our data (Fig. 1) support these findings and allow us to
257 investigate what remains poorly understood: the response and dynamics of microzooplankton
258 during these blooms and specifically whether microzooplankton grazing pressure aids in the
259 demise of blooms.

260 Smetacek (1981) first suggested that heterotrophic dinoflagellates and ciliates are
261 major herbivores in the spring and autumn. Since then, several studies have shown the
262 potential importance of microzooplankton during the development of the spring bloom (e.g.
263 the Gulf of Main, Montagnes et al. 1988; western Gulf of St. Lawrence, Canada, Tamigneaux
264 et al. 1997; the north-east Atlantic, Fileman and Leakey 2005; the eastern English Channel,
265 Grattepanche et al. 2011). However, we still lack an understanding of the role and dynamics
266 of microzooplankton during the development of the late summer/autumn bloom. Here, we
267 compare the two blooms and indicate clear differences in the composition and role of
268 microzooplankton.

269 Studies have also recognized species succession of microzooplankton during the
270 spring bloom (Fileman and Leakey 2005; Löder et al. 2011), but they have not assessed the

succession of the trophic roles of microzooplankton during the seasonal blooms. Given current recognition of the trophic diversity of microzooplankton (i.e. it includes herbivores, predators, and mixotrophs, Stoecker et al. 2017), we deemed it essential to obtain a better understanding of their changing role during blooms. Below, we first recognize shifts in the trophic succession of microzooplankton within and between blooms and then contrast the impact of microzooplankton as grazers between the two blooms, indicating a previously unappreciated impact during the late summer/autumn bloom.

Trophic succession of microzooplankton community composition

We reveal new patterns within the blooms that affect our appreciation of trophodynamics and nutrient export/recycling. In both blooms there was a general progression from auto- to heterotrophic processes, with predation playing a greater role near the end of the blooms (see Table S1 for the microzooplankton taxa and abundances). This might be expected, as initially light and nutrient conditions will likely benefit taxa such as red *Mesodinium* spp. which tend to rely more on phototrophy compared to phagotrophy according to their relatively low ingestion rates and long survival time without prey (Stoecker et al. 2017), while due to the lack of food, heterotrophs will be restricted. Then, as inorganic nutrients become limiting and prey become abundant, conditions are favourable for the mixotrophs, which obtain sustenance from ingestion, light, and dissolved inorganic nutrients (Jeong et al. 2010; Stoecker et al. 2017). We might also predict that due to their tropic flexibility, mixotrophs will sustain their abundance, which is supported by our data, where they remain more numerous than strict heterotrophs throughout both blooms, and beyond (Fig. 2). This agrees with arguments by Mitra et al. (2014) that mixotrophs will generally dominate the microzooplankton in more-mature systems, as the conditions in such systems normally favour mixotrophy due to the change of nutrients and particulate organic loading. The increase in mixotrophy will then lead

295 to greater recycling of inorganic nutrients (Hartmann et al. 2012), revising our understanding
296 of the rate at which nutrients decline due to export by sedimentation of large particles during
297 the progression of the spring and late summer/autumn blooms.

298 However, nutrients decrease as time progresses (Fig. 1), and strict heterotrophs
299 increase near the end of both blooms. The increase in heterotrophs may be explained by the
300 metabolic cost to mixotrophs, as they must maintain both auto- and heterotrophic processes
301 (Raven 1997); i.e. the maximum growth rates of heterotrophic microzooplankton are normally
302 higher than those of mixotrophs growing autotrophically (Jeong et al. 2010). Regardless of
303 why heterotrophs increase their appearance could lead to an increase in the export of materials,
304 assuming they are then grazed by the mesozooplankton populations that begin to develop near
305 the end of the spring bloom (Fig. 1). This argument is supported by Greve et al. (2004) who
306 reported that the small calanoid copepods dominate the mesozooplankton community at
307 Helgoland Roads, and in a mesocosm study at Helgoland, Löder et al. (2011) revealed that
308 dominant small calanoid copepod *Temora longicornis* exhibited selective feeding on
309 microzooplankton compared to phytoplankton. However, our most remarkable finding is the
310 increase in microzooplankton predators near the end of the bloom, indicating that strong
311 intraguild dynamics may structure the late-bloom trophodynamics (Fig. 2a,c). Such intraguild
312 predation within microzooplankton will likely lead to recycling rather than exporting nutrients
313 by sedimentation due to the small size of microzooplankton (Polis and Holt 1992), leading to
314 important changes in nutrient dynamics near the end of the bloom.

315 While the microzooplankton communities exhibited similar trends in trophic
316 composition, there were marked differences between the two blooms. The two most striking
317 difference were the persistence of red *Mesodinium* spp. and the greater, progressive increase
318 in predators in the late summer/autumn bloom (Fig. 2). Red *Mesodinium* spp. are obligate
319 mixotrophs and acquire plastids from specific prey (mainly the cryptophyte *Teleaulax*), by

which they can operate as phototrophs for some time (Stoecker et al. 2017). Though red *Mesodinium* spp. can maintain the plastids to some degree, they need to be replaced by consuming more prey (Hansen et al. 2013). Why red *Mesodinium* spp. persisted in the autumn is unclear, although it may be associated with surface cooling in early autumn (Johnson et al. 2013). Given that a similar phenomenon was not noted in the autumn bloom in the Gulf of Maine (Montagnes and Lynn 1989), the increase of red *Mesodinium* spp. may have been simply the occurrence of one extended bloom, similar to those that periodically occur throughout the summer and autumn (Fig. 1). In contrast, the progressive increase in predators may be of some note. Our more detailed species analysis revealed some notable observations: the mixotrophic, predatory dinoflagellates *Ceratium* spp. and *Dinophysis* spp. and the predatory ciliate *Tiarina fusus* - which were rare or absent in spring (Fig. 4a) - reached high abundances during the late summer/autumn bloom (Fig. 4b). Specifically, *Ceratium* spp. feed on ciliates (Smalley et al. 2003), *Dinophysis* spp. feed on *Mesodinium rubrum* (Park et al. 2006), and *Tiarina fusus* feeds on a variety of mixotrophic dinoflagellates (Jeong et al. 2002). Such details support the notion that the predatory interactions between dinoflagellates and ciliates are complex (e.g. Hansen 1991). For instance, in the late summer/autumn bloom, this interaction may reduce competition for inorganic nutrients or food resources by the consumption of competitors (Thingstad et al. 1996; Stoecker et al. 2017). Regardless, it appears that there is a propensity for even greater intraguild interactions during the late summer/autumn bloom. How these interactions alter dynamics within the late summer/autumn bloom is clearly an area for further detailed investigation.

Effects of top-down control by microzooplankton during the blooms

Microzooplankton grazing is predicted to remove 60 to 75% of the primary production across a spectrum of open oceans and coastal systems (Landry and Calbet 2004), and at times they

may exert significant top down control, consuming over 100% of primary production (Löder et al. 2011). Here we assess if their grazing pressure might control the spring and late summer/autumn blooms.

Across the spring bloom microzooplankton grazing was relatively low, at best removing ~42% primary production and ~27% of the standing stock (Fig. 3c, d). Surprisingly, growth rates were relatively high (Fig. 3e), but this may have resulted from the high level of mixotrophy (Fig. 2a, b). Collectively these data suggest that although microzooplankton grazing may have contributed to its control, the decay of the spring bloom was unlikely to have been caused only by their grazing when top-down control by mesozooplankton is lacking as has been suggested (Löder et al. 2011). Rather, as is classically argued, the decay of the spring bloom is likely due to both the limitation of nutrients and increased mesozooplankton grazing pressure (e.g. Fig. 1a, c; Löder et al. 2011). Accordingly, it seems that although microzooplankton play an important role in consuming and recycling autotrophic production in the spring, they may not (always) be responsible for causing the demise of bloom.

In contrast, microzooplankton likely contribute to the control of phytoplankton populations and play a role in the demise of the late summer/autumn bloom. During the late summer/autumn bloom, microzooplankton grazing and growth rates were substantially higher than those in the spring (Fig. 3), with more than 100% of the primary production and well over 100% of the standing stock being consumed (Fig. 3h, i) towards the end of bloom. At this time, copepod abundance is relatively low, suggesting a reduced grazing pressure by mesozooplankton (Fig. 1c). Furthermore, the high abundance of microzooplankton, especially dinoflagellates near the end of the late summer/autumn bloom suggests they will have substantial impact (Fig. 1b, e). Clearly, decreasing light levels in the autumn will contribute to reduced phytoplankton growth and hence the reduction of the bloom (e.g.

Longhurst 1995), but light levels in our spring and late summer/autumn experiments were similar, suggesting that this was not a confounding factor in this study when comparing the relative contribution of microzooplankton to bloom demise. In contrast, late summer/autumn temperatures at our study site tended to be higher than those in the spring. As growth and grazing rates of microzooplankton are likely to increase with temperature (e.g. Wang et al. 2019), this could add to their higher impact in the autumn. Based on these observations, we suggest that microzooplankton aid substantially (e.g. Fig 3) in the termination of the late summer/autumn bloom.

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Legends:

Fig. 1 Annual patterns of nutrients, chlorophyll *a*, phytoplankton, microzooplankton, and copepods in the waters around the island of Helgoland

Fig. 2 Abundances and proportion of different functional groups in the microzooplankton communities at different stages of the spring bloom and late summer/autumn bloom

Fig. 3 Estimates of the grazing mortality coefficients (g, d^{-1}), phytoplankton growth coefficients (k, d^{-1}), daily percent loss of phytoplankton standing crop (P_i), daily percent loss of phytoplankton production (P_p), and growth rates of microzooplankton during the stages of blooms. The dash lines indicate the average grazing impact of P_p by microzooplankton across a spectrum of open oceans and coastal systems proposed by Landry and Calbet (2004), and the error bars correspond the standard errors with *p* and *df* values reflecting the test to determine if the slope differed from zero ($\alpha = 0.05$)

Fig. 4 Total abundances of the mixotrophic dinoflagellates *Ceratium* spp. and *Dinophysis* spp. and the ciliated predator *Tiarina fusus* at different stages of the spring bloom and late summer/autumn bloom

526 **Table 1** Field conditions when dilution experiments were conducted

	Spring			Late Summer/Autumn		
	Pre-bloom	**Bloom**	**Post-bloom**	**Pre-bloom**	**Bloom**	**Post-bloom**
Date (2012)	04-Apr	25-Apr	09-May	01-Aug	15-Aug	22-Aug
Temperature (ºC)	6	7	9	16	17	18
Light cycle (h)	7:00-20:00	6:00-21:00	5:30-21:30	6:00-21:30	6:00-21:30	6:30-21:00
Chl *a* (μg L^{-1})	1.43	6.15	0.91	1.86	4.09	2.65
Diatoms (L^{-1})*	2.38×10^4	1.12×10^5	1.32×10^4	8.56×10^4	1.26×10^6	1.61×10^5
Phytoflagellates (L^{-1})*	1.48×10^6	7.83×10^5	1.46×10^5	2.13×10^6	4.60×10^6	1.15×10^6

527 *mean abundances of diatoms and phytoflagellates in undiluted treatments of each experiment before

528 incubation.

Figure 1

Click here to access/download;Figure;Fig. 1.tif

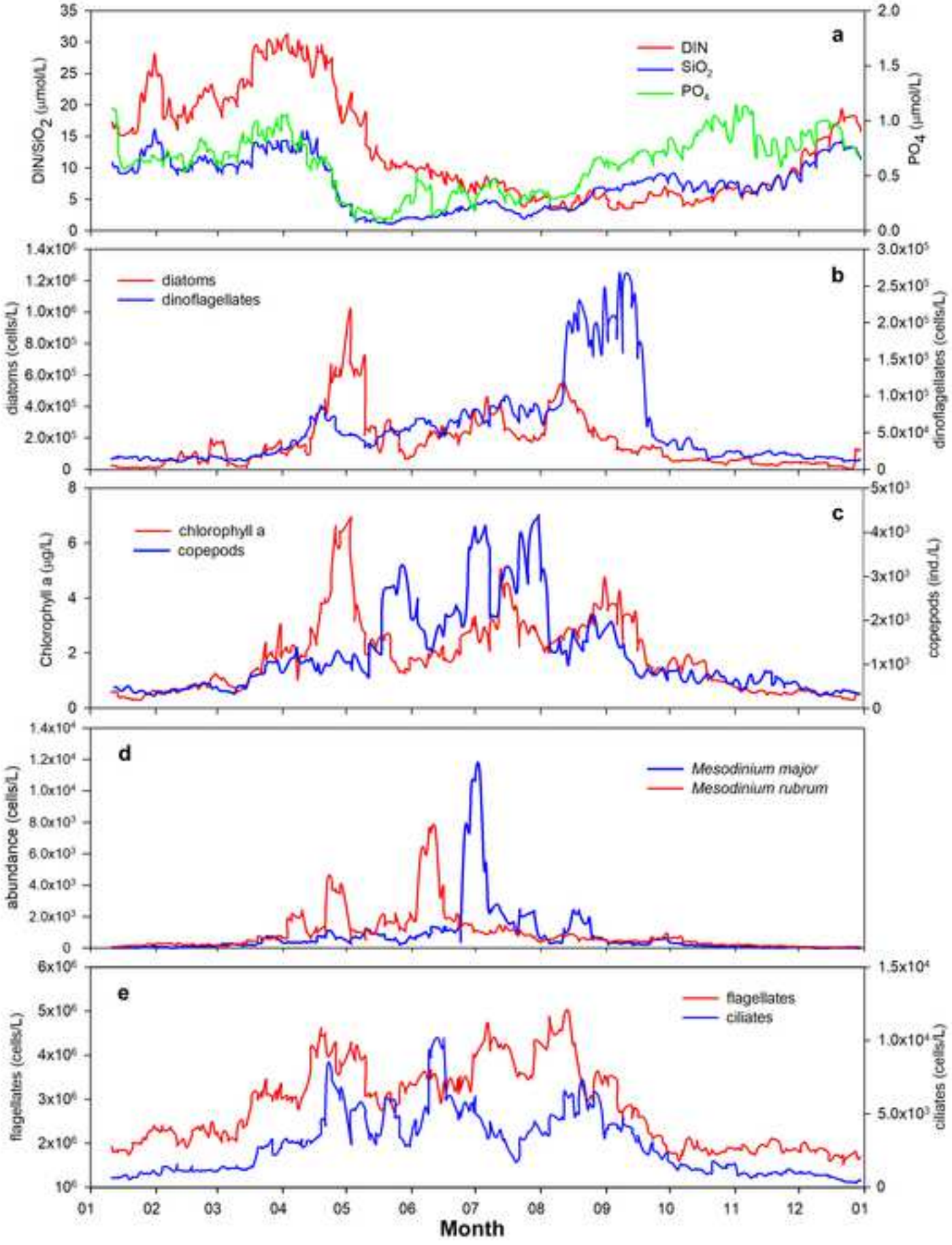


Figure 2

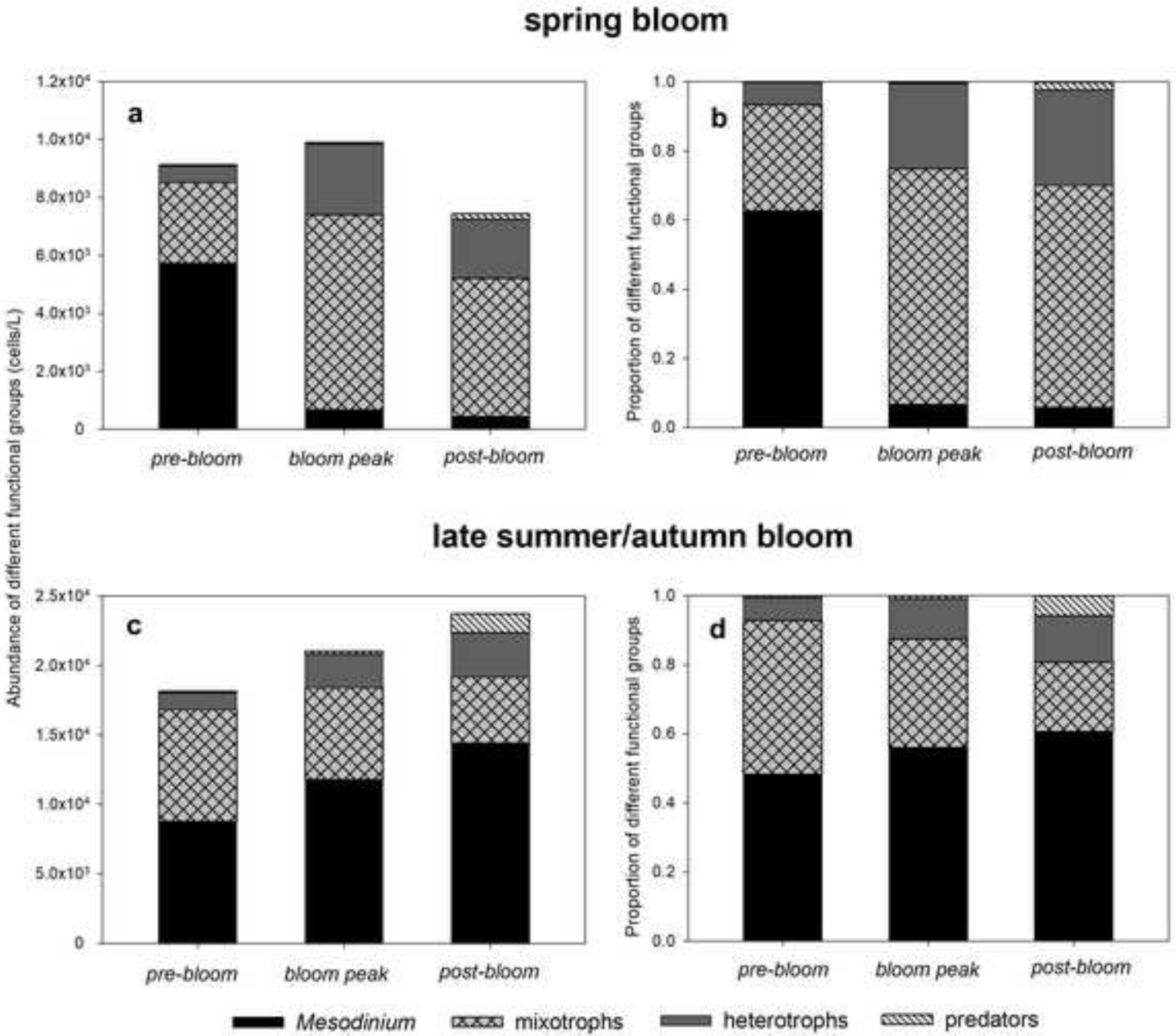


Figure 3

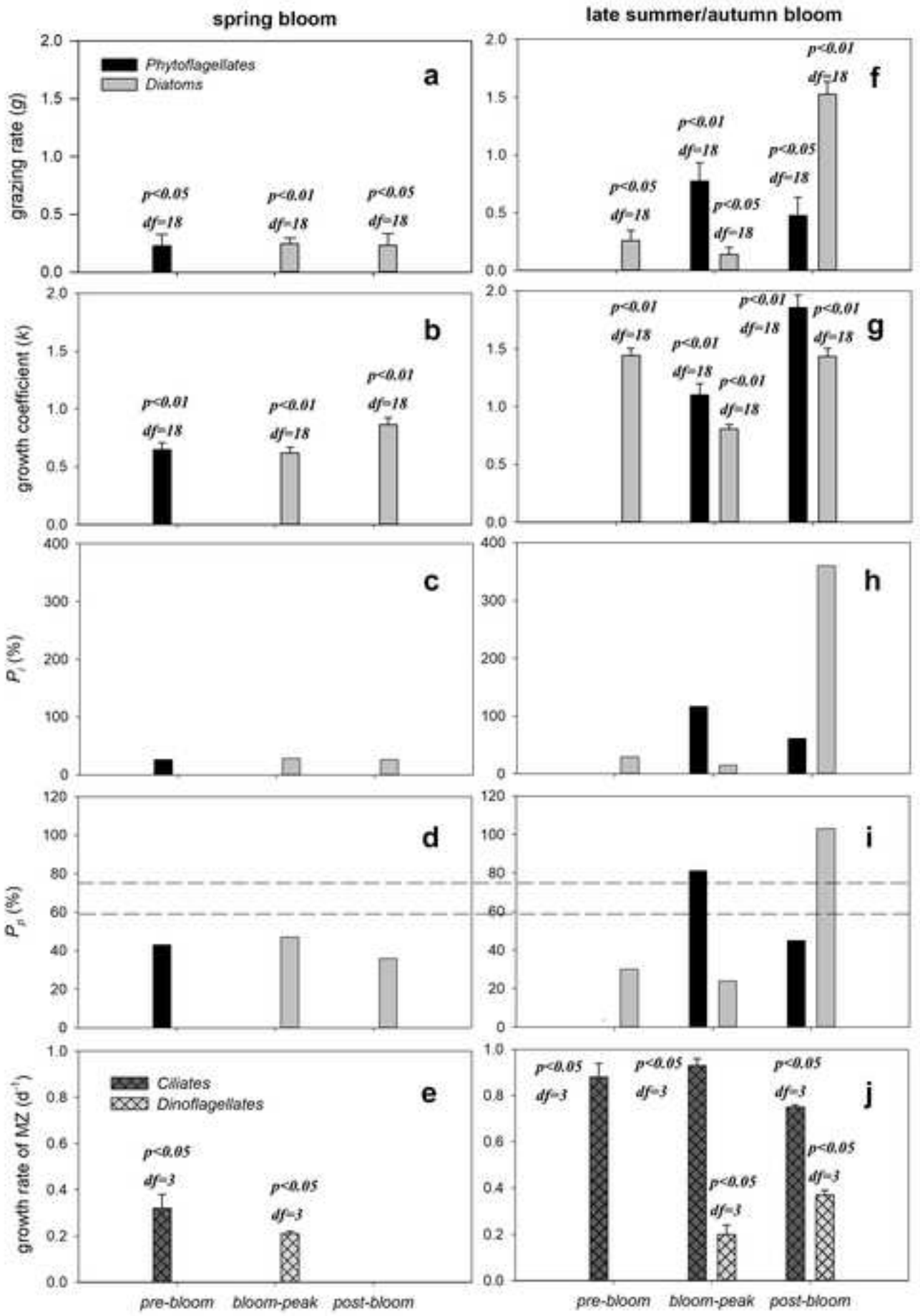
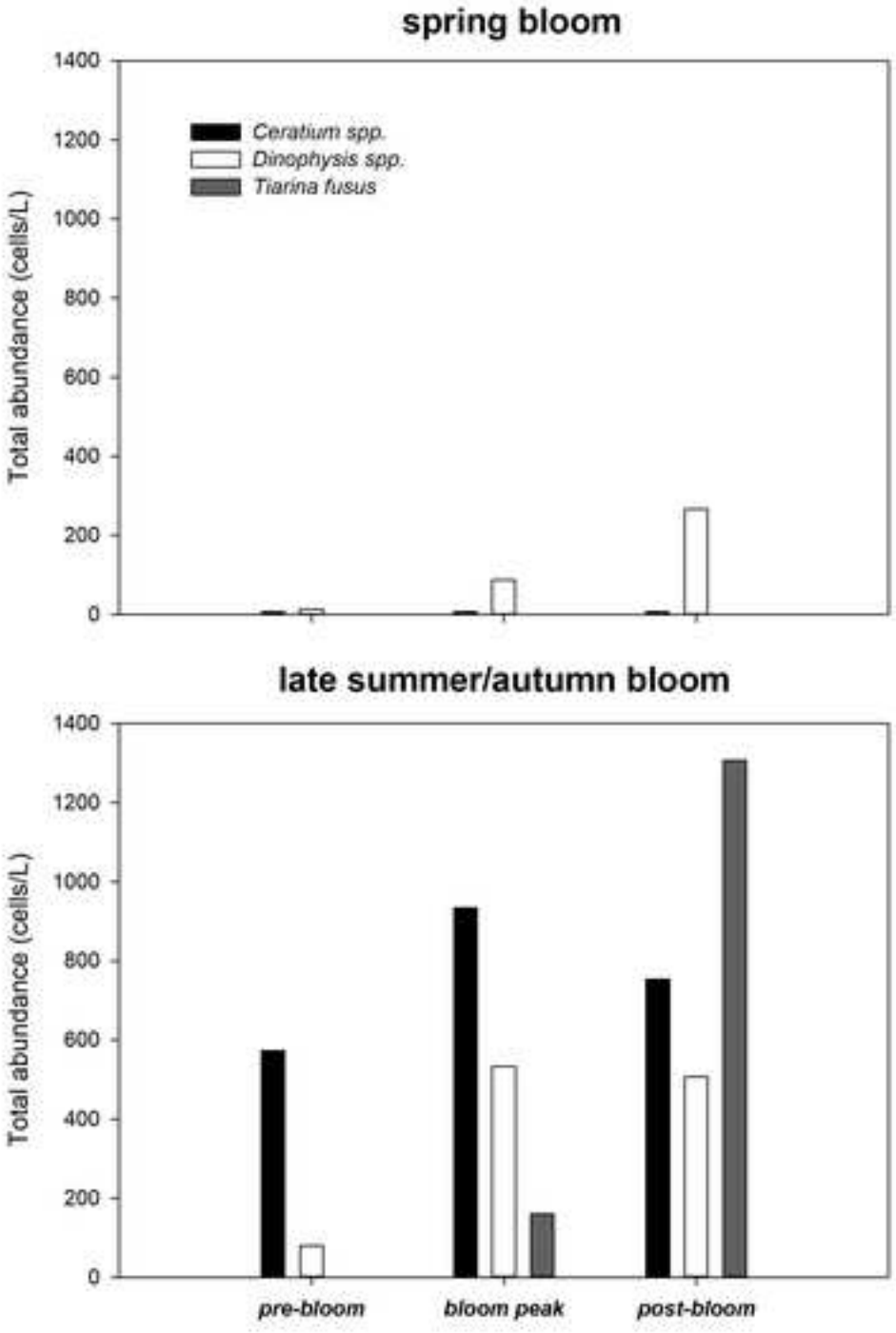


Figure 4



Supplementary Material including supplemental information on Methods, Fig. S1 and Table S1

Article Title: Comparing the trophic impact of microzooplankton during the spring and autumn blooms in temperate waters

Journal Name: Estuaries and Coasts

Author Names: Jinpeng Yang, Martin Günter Joachim Löder, Karen Helen Wiltshire and David Montagnes

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Supplemental information concerning "Identifying annual patterns at Helgoland" in the Materials and Methods:

Surface water samples were taken from the Helgoland Roads long-term station "Kabeltonne" on a daily to weekly basis (see Appendix 1 for details of dates)

For ciliates, a 250 ml sample was fixed with acid Lugol's solution (2% final concentration) and stored at 4 °C in the dark. Then, a 50 ml subsample was settled in a Utermöhl chamber for at least 24 h. The whole chamber was examined at 200-fold magnification under an inverted microscope (Zeiss Axiovert 135). Following this procedure, ~ 20 individuals of the most abundant taxa were observed. Some ciliates were only identified to genus level or placed into size groups or morphotypes, as Lugol's obscures some diagnostic characters.

The data for environmental variables (DIN, phosphate, silicate, and Chlorophyll a), phytoplankton, and dinoflagellates were obtained from the routine monitoring at the "Kabeltonne" site, where biological, chemical and physical parameters are recorded continuously on a work-daily basis (Franke et al. 2004). Following similar methods as described for ciliates above, the phytoplankton and dinoflagellate species numbers were counted to species level wherever possible. Only those data corresponding to the date of collection for ciliate data were used in our analysis (Appendix 1).

Mesozooplankton determined to species level have been included into the routine monitoring at the "Kabeltonne" site since 1975 (Greve et al. 2004). Samples were collected using a HYDROBIOS quantitative collection hand-net (mesh size 150 µm, net length 100 cm) on a daily to weekly basis. Copepod data used in our analysis (Appendix 1) were taken from the mesozooplankton dataset and the taxa included *Acartia* spp., *Alteutha interrupta*, *Amphiascus* sp., *Calanus* spp., *Centropages* sp., *Corycaeus anglicus*, Cyclopoidea, *Ectinosoma* sp., *Euterpina acutifrons*, *Harpacticoida* sp., *Microcalanus* sp., *Microsetella* sp., *Monstrilla helgolandica*, *Monstrilla* sp., *Oithona* sp., *Oncaea similis*, *Parathalestris* sp., *Pseudo-/Paracalanus* spp., *Temora longicornis*, *Thalestris longimana*, *Tisbe* sp., and nauplii.

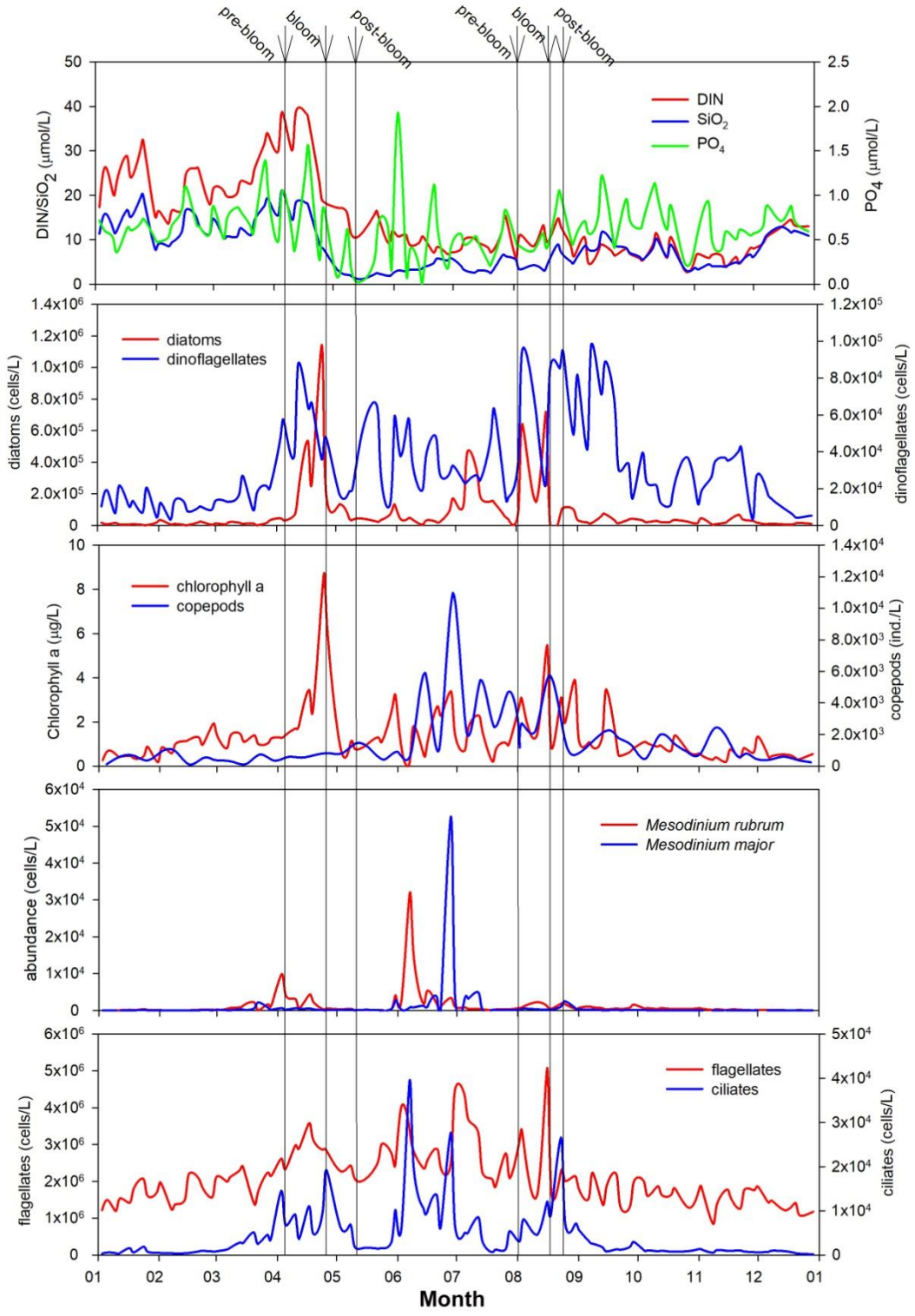

Fig. S1 Annual variations of nutrients, chlorophyll *a*, phytoplankton, microzooplankton, and copepods in the waters around the island of Helgoland in 2012. Dates when microzooplankton trophic composition and grazing rates were determined are marked with arrows (Table 1 provides details)

Table S1 Average abundances (cells L^{-1}) of ciliates and dinoflagellates in the undiluted treatments of grazing experiments during the blooms at Helgoland Roads.

Species	spring bloom	late summer/autumn bloom
red *Mesodinium* spp.		
Mesodinium rubrum	1987	2016
Mesodinium major	284	9622
Mixotrophs		
Cyrtotrombidium sp.	0	36
Laboea strobila	173	22
Strombidium cf. *capitatum*	11	29
Strombidium cf. *emergens*	373	0
Strombidium cf. *epidemum*	269	0
Strombidium cf. *lynii*	5	20
Strombidium cf. *stylifer*	18	42
Strombidium cf. *sulcatum*	244	589
Strombidium sp. 15µm	993	1116
Strombidium sp. 20µm	255	82
Strombidium sp. 35µm	0	384
Strombidium sp. 50µm	2	0
Strombidium sp. 100µm	15	0
Tontonia cf. *gracillima*	158	18
Ceratium furca	2	642
Ceratium fusus	5	60
Ceratium horridum	0	1718
Dinophysis sp.	122	373
Heterocapsa sp.	1907	55

Torodinium sp.	42	382
Heterotrophs		
Balanion sp.	7	180
Eutintinnus sp.	0	33
Holophrya vorax	0	60
Leegaardiella cf. *ovalis*	73	0
Leegaardiella cf. *sol*	2	0
Lohmaniella oviformis	107	0
Pelagostrobilidium cf. *neptunii*	7	80
Rimostrombidium sp.	0	2
scuticociliates	55	51
Strobilidium sp. 15µm	0	11
Strombidinopsis sp.	0	7
Tintinnidium cf. *balechi*	5	0
Tintinnopsis cf. *parvula*	20	7
Tintinnopsis sp.	7	0
Diplopsalis sp.	140	102
Gyrodinium cf. *spirale*	1218	1518
Gyrodinium sp.	5	13
Katodinium sp.	38	824
Protoperidinium sp.	187	309
Predators		
Askenasia regina	58	36
Didinium sp.	7	0
Placus sp.	13	36
Tiarina fusus	0	489

Appendix 1 including the original data

date	DIN	PO4	SiO2	Chla	ciliates	M. rubrum	M. major	flagellates
1/23/2007	38.422	0.6518	21.8304		2140	0	40	2458067
1/30/2007	39.4974	0.6721	24.1389		1360	60	20	2453378
2/6/2007	34.3589	0.6721	14.859		880	20	0	2812625
2/13/2007	29.7397	0.3583	16.049		920	0	0	2743954
2/20/2007	25.7295	1.2389	13.4494		820	160	80	3265009
2/27/2007	18.6254	0.8543	10.2319		720	40	20	2834415
3/6/2007	23.2171	0.7834	12.7702		860	120	100	2172123
3/13/2007	28.4439	1.6032	13.8223		1140	160	160	2616571
3/20/2007	36.9122	1.3704	16.4116		9760	560	840	2756289
3/27/2007	45.239	1.0567	21.9326		5380	560	340	3486307
4/3/2007	44.4814	1.2389	19.0981		3080	440	160	2992056
4/10/2007	37.6852	1.8057	37.2033		4440	400	680	3588954
4/17/2007	42.0658	0.8644	19.6088		4880	1240	280	3015491
4/24/2007	32.9097	0.3381	8.8631		30680	24960	3320	3978532
5/3/2007	47.114	0.1761	5.0072		7360	3400	40	6709557
5/10/2007	21.512	0.0547	1.2993		4760	200	440	5125611
5/15/2007	19.0336	0.01	0.4413		2880	340	20	4569893
5/22/2007	11.8866	0.01	0.8703		6280	2360	120	2535814
5/29/2007	13.6809	0.4494	1.0746		2640	520	420	2515815
6/5/2007	11.5203	0.0294	0.1885		940	300	1420	6379376
6/12/2007	6.2024	0.0951	0.9877		6160	1000	2800	4872185
6/19/2007	4.8598	0.0344	1.9581		3520	1160	600	4270648
6/26/2007	1.016	0.01	0.1859		3780	720	1240	3894483
7/3/2007	4.4107	0.8644	4.1389	0.777	1340	320	2060	3794999
7/10/2007	4.9237	0.5354	4.3866	2.4555	6303	840	400	4512483
7/17/2007	3.6817	0.6215	3.5209	2.189	1620	680	820	3146738
7/24/2007	1.1168	0.3583	1.1716	4.7715	9820	360	320	5127841
7/31/2007	2.5538	0.247	3.5669	1.4865	4450	280	280	5110716
8/7/2007	1.5858	0.4291	2.1062	1.933	12980	1000	400	4556452
8/14/2007	0	0.4393	2.3514	5.282	16760	1040	240	4035685
8/21/2007	0	0.6012	6.0592	3.633	14220	400	80	2606680
8/28/2007	0.1287	0.5506	7.4229	1.9865	12960	1040	120	5524958
9/6/2007	0.4879	0.8745	9.2717	1.4745	4280	240	1080	2333841
9/13/2007	2.2824	0.8543	9.7671	1.3155	3180	200	480	4120020
9/18/2007	2.6053	0.8846	11.1154	2.2363	4110	160	80	2675111
9/25/2007	1.3632	1.0466	10.9111	3.157	3240	200	560	2612572
10/2/2007	1.5168	0.8441	12.3667	3.8665	3040	280	120	2605599
10/9/2007	4.5716	1.2794	13.8478	5.784	3180	0	200	3094238
10/16/2007	1.9731	0.9251	11.1614	0.666	3210	40	200	1450173
10/23/2007	14.0592	1.6538	18.4545	1.115	6306	180	80	2012735
10/30/2007	6.3991	1.1174	11.9173	0.7715	2570	60	0	1893293
11/6/2007	10.8914	1.502	14.8386	1.197	1340	60	60	2269401
11/13/2007	14.9185	2.0081	16.7896	0.915	1860	0	60	2757094
11/20/2007	14.8299	1.2996	16.5802	0.322	2340	120	20	2335390
11/27/2007	17.3023	1.836	16.5802	0.6185	2556	0	400	2366515
12/4/2007	16.2978	1.5729	16.999	0.577	1510	20	0	2299651
12/11/2007	13.9467	1.6538	15.763	0.3345	953	20	20	2673995
12/18/2007	20.4288	0.9328	16.4861	0.2655	700	0	60	2397729
12/27/2007	15.2374	0.8137	14.6937	0.1925	260	120	0	2371708

1/3/2008	15.4927	0.6807	11.7137	0.2585	360	180	0	2360982
1/8/2008	12.5324	0.6106	6.7913	0.305	2543	180	60	2805599
1/15/2008	11.8627	0.8347	6.5361	0.4715	840	220	0	2237971
1/22/2008	14.112	0.8347	7.2852	0.341	760	120	0	1644833
1/29/2008	17.5036	0.9888	14.283	0.577	1500	200	400	2543104
2/5/2008	15.4607	1.563	11.8135	0.605	880	60	0	2463842
2/12/2008	14.0442	0.7087	7.4573	1.4905	820	20	200	2906781
2/19/2008	29.6916	0.9818	16.1698	0.584	1460	220	160	3076192
2/26/2008	26.3672	0.8207	11.1032	2.3595	2000	100	60	3146126
3/4/2008	30.686	1.472	13.4506	0.5955	2280	80	20	3490966
3/11/2008	35.7201	1.2549	16.6415	0.564	1240	200	80	3016361
3/18/2008	58.3849	1.612	31.7969	0.949	2080	200	80	4106986
3/25/2008	37.4442	1.0308	18.9445	0.8135	1880	60	40	2472662
4/1/2008	31.5055	0.8697	12.5294	0.779	5780	520	40	2354548
4/8/2008	33.5823	8.0756	13.4118	1.535	6320	560	340	2410153
4/15/2008	25.9415	0.4496	7.424	1.2405	1820	840	40	3136861
4/22/2008	9.0181	0.2255	2.141	3.4435	3160	500	260	3095735
4/29/2008	4.6072	0.2325	1.3141	4.8455	2060	600	380	4455343
5/6/2008	1.5105	0.2885	2.5183	4.4995	3000	140	420	3993708
5/13/2008	6.4194	0.3235	2.9623	5.1645	3720	120	60	4132581
5/20/2008	7.9355	0.1975	1.1365	1.859	4720	20	100	4302053
5/27/2008	7.2101	0.2815	3.3951	1.3955	7500	560	180	5097722
6/3/2008	2.3943	0.2465	1.5694	2.7055	13000	920	360	3343646
6/10/2008	0.5629	0.2675	0.8535	1.652	31480	3800	320	4103112
6/17/2008	0.3223	0.3866	0.9756	1.763	5743	2780	2480	4539127
6/24/2008	0.1032	0.1415	1.5749	1.3385	2867	1360	1440	5626883
7/1/2008	0.4481	0.2255	2.5519	1.277	1560	80	2160	2749146
7/8/2008	0.3273	0.1765	3.5738	1.6985	2160	100	300	3798790
7/15/2008	8.7229	0.2115	4.9344	1.2115	1060	120	2480	5047331
7/22/2008	0.0637	0.3235	0.2404	4.927	3500	840	2680	5321258
7/29/2008	2.4598	0.2115	2.7213	1.5895	4740	120	2220	7673083
8/5/2008	2.0089	0.3305	4.2404	1.3275	4240	140	80	7393049
8/12/2008	2.6	0.5546	4.9781	1.8405	4020	100	380	5743146
8/19/2008	1.4048	0.6807	6.0546	2.114	6540	0	60	3141799
8/26/2008	2.3081	0.5336	7.6776	1.313	2000	20	40	3004055
9/2/2008	2.4121	0.7927	9.0437	1.007	6560	60	120	3278389
9/9/2008	3.4647	0.6877	9.1475	0.575	1800	280	80	2647571
9/16/2008	1.5956	0.3936	3.6066	1.2435	7140	1400	320	2288851
9/23/2008	1.1934	0.3866	3.3716	1.3065	6660	2340	1620	2876688
9/30/2008	0.4783	0.3936	2.1803	1.6085	2997	1480	960	307830
10/7/2008	2.1027	0.5896	3.9454	0.23	3580	940	180	2725942
10/14/2008	3.0931	0.7717	5.623	0.9865	700	260	100	2795819
10/21/2008	3.1583	0.7717	6.1639	1.2655	1313	180	40	2102559
10/28/2008	3.3329	0.9958	5.2131	0.5225	937	40	20	2408351
11/4/2008	2.8641	0.7437	5.5301	0.334	600	40	80	1752733
11/11/2008	1.7988	0.5126	3.7814	0.5125	1640	40	0	2551367
11/18/2008	3.0523	0.5826	6.3443	0.586	1260	100	0	2592492
11/25/2008	17.6958	1.0308	15.5191	0.388	1657	20	0	2789837
12/2/2008	4.8559	0.5826	4.9563	0.2485	920	80	40	1315836
12/9/2008	6.2283	0.8067	9.4153	0.312	1153	40	0	2158667

12/15/2008	7.4146	0.8487	6.4481	0.3845	480	0	0	498613
12/23/2008	10.6341	0.6036	7.9563	0.5015	640	20	0	1416645
12/30/2008	9.9449	0.6387	6.3607	0.3215	1717	20	0	1831050
1/6/2009	15.4	0.9	10.93	1.31	640	0	0	1917291
1/13/2009	9.35	0.53	7.58	0.183	600	80	40	1785684
1/20/2009	8.84	0.53	5.78	0.185	1330	60	20	2118701
1/28/2009	7.28	0.5	4.1	0.514	760	80	100	2598427
2/3/2009	5.78	0.41	4.07	0.804	3680	340	0	2810711
2/10/2009	7.65	0.36	3.29	0.261	3980	60	80	119833
2/17/2009	8.25	0.37	2.93	0.444	2580	20	40	2604732
2/25/2009	11.72	0.58	5.56	0.31	2340	240	60	2014613
3/3/2009	10.24	0.29	5.4	0.164	2700	180	120	476240
3/10/2009	10.05	0.19	2.58	0.37	1220	140	180	1786187
3/17/2009	12.29	0.29	3.15	4.521	1737	300	100	5390817
3/24/2009	15.93	0.01	1.05	8.025	2697	1060	600	5648881
3/31/2009	12.45	0.01	0.01	9.291	3937	340	40	4662118
4/9/2009	7.92	0.01	0.01	5.473	3697	40	40	4420162
4/14/2009	8.37	0.01	0.17	3.621	4120	40	120	5555323
4/21/2009	8.85	0.06	1.17	2.0195	9880	440	140	2859401
4/28/2009	8.48	0.01	0.57	0.418	2500	800	220	2476403
5/5/2009	10.6045	0.1871	1.459	3.9805	13890	2640	2480	2896531
5/12/2009	11.5161	0.0504	2.5956	0.7025	3320	5100	1260	2800979
5/19/2009	6.804	0.0863	1.7268	0.9335	1820	8060	3080	2339391
5/26/2009	6.1969	0.01	1.9508	2.8325	1653	5420	1300	3292620
6/2/2009	10.2766	0.1439	2.2459	0.6565	2073	2700	0	2639916
6/9/2009	4.3421	0.01	1.0328	4.123	1447	3320	140	3514576
6/16/2009	2.7706	0.0288	0.612	3.091	8143	1940	120	5075138
6/23/2009	3.29	0.1439	0.8907	1.8735	3537	3680	700	4269506
6/30/2009	4.0173	0.0719	1.5355	4.99	6514	340	23560	8149309
6/8/2010	16.1246	0.1366	3.9322	2.3605	12067	2220	1920	1665200
6/10/2010	13.4734	0.0888	4.16	2.3455	10726	1467	2460	1747201
6/15/2010	15.2518	0.3484	4.9439	1.962	6041	200	207	2499085
6/17/2010	16.1817	0.4098	5.1239	1.594	7070	500	290	1641708
6/22/2010	13.0328	0.485	5.929	2.4055	2408	133	87	2540540
6/24/2010	12.4137	0.1571	5.2511	5.488	9826	247	140	2282719
6/29/2010	14.8729	0.362	6.9301		3780	1127	260	2465733
7/1/2010	15.6918	0.123	7.1949		10914	1700	1173	5238466
7/6/2010	14.8629	0.5806	6.125		6268	2540	3840	4395836
7/8/2010	8.2145	0.0751	4.9386	6.7345	6068	2227	3593	4618394
7/13/2010	4.5004	0.01	0.9767	14.54	2840	3993	2200	4466944
7/15/2010	3.622	0.01	1.6176	4.5925	1653	2633	913	3834459
7/20/2010	3.7348	0.0273	1.8824	2.6195	2704	1367	1393	3318092
7/22/2010	2.7697	0.1913	1.321	5.243	5994	2773	10747	3906944
7/27/2010	1.3736	0.1161	1.2892	3.189	5726	587	2127	3776084
7/29/2010	2.2824	0.1093	2.2426	2.075	3206	667	347	3996077
8/3/2010	2.3225	0.1708	3.5085	2.905	6126	640	327	4653213
8/5/2010	3.1564	0.1161	2.7246	3.748	1639	133	47	3916748
8/10/2010	3.1154	0.123	3.2119	3.8635	4766	753	1047	4810773
8/12/2010	3.1285	0.0342	3.2436	3.766	5875	480	6420	4486350
8/17/2010	4.2265	0.2117	4.4725	4.1625	3560	280	9467	3819120

8/19/2010	4.5466	0.2801	4.6102	3.6565	1780	100	1827	4510163
8/24/2010	3.279	0.3074	5.6695	7.57	1553	0	107	4423494
8/26/2010	3.5659	0.9085	7.1472	6.33	1699	53	40	4721962
8/31/2010	2.6541	0.3552	4.5784	11.2965	3748	80	93	3975150
9/2/2010	3.4408	0.3962	5.9502	2.281	3300	133	127	3636834
9/7/2010	1.4178	0.2664	3.0106	10.9925	10701	360	107	2995095
9/9/2010	2.3816	0.526	6.3475	7.947	7980	133	347	3283365
9/14/2010	4.7027	0.5123	5.59	1.3205	1853	160	413	3830274
9/16/2010	5.5803	0.5943	7.0625	0.7135	2167	27	340	1358912
9/21/2010	10.8671	0.8128	9.9597	1.0755	866	100	540	2237598
9/23/2010	9.2232	0.6079	10.5159	1.18	1953	333	1227	2015151
9/28/2010	9.7078	0.8538	9.5519	1.596	627	80	367	1301171
9/30/2010	12.2732	1.209	12.1525	1.3275	627	113	227	1970851
10/5/2010	1.8563	0.2459	4.0593	0.823	347	287	427	1463720
10/7/2010	1.8241	0.5601	3.8633	0.903	473	167	373	1188453
10/12/2010	2.1708	0.9973	3.3496	2.0855	860	120	200	1463423
10/14/2010	3.3853	0.6011	3.2225	1.17	554	280	407	1152784
10/19/2010	3.7964	1.9809	2.3485	0.9305	1047	180	173	2050594
10/21/2010	4.5485	0.7104	3.1907	0.8275	408	40	147	1746086
10/26/2010	9.8777	0.7104	7.179	0.7305	228	80	140	1700839
10/28/2010	8.5779	0.7309	6.7553	0.9045	393	80	160	2048535
11/2/2010	4.5195	3.2582	5.2193	0.6205	721	107	67	2008707
11/4/2010	4.9087	0.4645	5.2617	0.525	527	193	607	1965350
11/9/2010	7.3245	0.5328	5.2405	0.5955	328	120	53	1958082
11/11/2010	5.5553	0.3552	5.0498	0.666	230	207	87	1981315
11/16/2010	11.2826	0.5396	8.7468	0.3235	233	127	0	2244356
11/18/2010	5.9042	0.5806	6.1992	0.4345	198	107	53	1401330
11/23/2010	3.4806	0.3074	3.3919	0.827	840	73	20	1220201
11/25/2010	7.0824	0.4303	6.035	0.3565	174	187	33	1472038
12/2/2010	25.0858	0.6694	15.4364	0.7555	554	87	7	2209906
12/9/2010	29.7484	0.8402	18.1854	0.663	307	80	0	1523462
12/16/2010	34.4109	1.0109	20.9343	0.5705	381	87	0	1819252
12/21/2010	21.7559	0.6284	11.1091	0.171	221	100	7	1798391
12/22/2010	15.8738	0.5533	11.0297	0.043	147	60	20	1950184
12/28/2010	30.5434	0.929	18.4555	1.945	147	147	7	1910939
12/30/2010	17.9936	0.5943	10.2511	0.464	154	100	7	1703318
1/4/2011	16.4398	3.0601	10.4364	0.7845	187	60	0	2073407
1/6/2011	14.3323	1.8101	7.5869	0.5645	121	67	7	2275026
1/11/2011	7.1047	0.5874	5.3835	0.1595	348	160	0	1361922
1/13/2011	10.1992	0.526	6.1515	0.413	206	167	7	2310695
1/18/2011	13.7517	0.7036	9.2447	0.1175	400	173	0	2034250
1/20/2011	17.3822	0.5123	8.3602	0.1425	234	247	0	2364552
1/25/2011	16.7456	0.485	7.9047	1.077	560	413	13	2564433
1/27/2011	44.8721	0.6421	19.0593	0.343	628	700	13	2882766
2/1/2011	25.2298	0.6148	14.7479	0.3035	1140	780	20	2205701
2/3/2011	15.5125	0.5396	9.679	0.7925	1867	660	20	2162834
2/7/2011	19.3598	0.4713	9.2765	0.272	574	367	13	2793683
2/10/2011	24.0912	0.5464	12.0254	0.272	1301	1200	40	2675640
2/15/2011	17.0369	0.5123	6.6494	0.383	995	380	73	2893615
2/17/2011	20.6127	0.9563	9.8273	0.3605	1614	553	240	757654

2/22/2011	21.6457	0.4713	8.0847	1.6335	767	187	120	2436071
2/24/2011	19.6998	0.362	7.3591	0.2085	1019	240	260	1760412
3/1/2011	19.5438	0.3962	7.6716	0.92	1059	67	693	2386945
3/3/2011	23.1475	0.3893	8.4025	0.1035	1215	227	933	2167891
3/10/2011	23.2386	0.5738	8.4979	0.4235	1658	300	487	2846396
3/15/2011	30.6184	0.724	12.804	0.411	1141	73	213	2778809
3/17/2011	22.9588	0.5738	8.9057	0.24	1621	247	280	2787086
3/22/2011	21.9915	1.2158	10.5212	0.5055	2000	167	307	3859588
3/24/2011	23.8974	0.9904	9.5095	0.4695	1814	374	1120	2941835
3/29/2011	29.943	1.7691	11.9089	0.473	1768	233	227	3287241
3/31/2011	21.0145	1.1475	11.9248	0.3845	2107	160	953	3524062
4/5/2011	22.4313	0.5943	9.0381	0.7915	1559	193	360	2851613
4/7/2011	20.6446	0.8743	9.5625	0.9815	1108	260	440	3129459
4/12/2011	27.8871	0.5533	12.4597	1.122	430	210	200	4216894
4/14/2011	19.4893	0.5738	7.5869	4.519	1245	320	913	6824604
4/19/2011	26.453	0.3689	6.2733	9.307	6179	307	3227	7607024
4/21/2011	26.6413	0.3415	4.9121	4.74	2884	707	540	5416114
4/26/2011	16.6316	0.123	0.6324	15.425	2494	187	300	5332954
4/28/2011	23.8682	0.01	0.0657	6.0485	4914	473	600	5060722
5/3/2011	18.2657	0.0751	0.1398	5.061	3306	520	673	4412524
5/5/2011	18.9343	0.01	0.01	1.0315	2056	33	1133	3950745
5/10/2011	11.3623	0.01	0.3729	1.7035	2583	713	3807	1804378
5/12/2011	9.8151	0.01	0.3623	3.542	2841	680	1580	2238748
5/17/2011	10.4585	0.2322	0.5636	5.266	10797	1047	373	2495123
5/19/2011	6.6321	0.0137	0.6377	1.2095	17315	947	387	2720852
5/24/2011	9.2274	0.0615	1.9619	0.1145	3289	767	40	2543778
5/26/2011	8.9268	0.1708	1.7288	0.7395	2596	387	47	3790421
5/31/2011	11.0157	0.123	1.9778	0.9565	1668	3820	527	3972583
6/3/2011	10.7547	1.93	3.0689	0.747	2900	3420	127	4053338
6/7/2011	11.1784	0.2049	2.9153	0.1655	6726	31760	813	3419680
6/9/2011	8.8507	0.3893	3.2489	1.8095	4581	15093	820	2838353
6/14/2011	9.4989	0.1708	3.2595	0.56	7549	1893	1267	2360982
6/16/2011	10.6218	0.1161	3.821	1.052	3342	5387	960	2552079
6/21/2011	7.1691	1.0929	4.6896	2.701	8533	1420	3440	2881484
6/23/2011	8.4258	0.6557	5.8178	2.286	3723	1160	1787	2346175
6/28/2011	6.8536	0.1776	5.3464	3.387	11640	3420	52640	2483167
6/30/2011	7.1091	0.4235	5.8761	1.458	1635	1000	4407	4455998
7/5/2011	7.8173	0.5669	4.197	5.476	945	833	3213	5248466
7/7/2011	10.1822	0.444	3.1589	1.759	1607	420	3140	3672238
7/12/2011	10.3442	0.5328	2.6186	2.293	3245	393	4920	3328434
7/14/2011	9.3215	0.4713	3.1112	1.602	2635	313	273	4347569
7/19/2011	8.1961	0.2459	2.8623	0.2215	435	87	133	2063392
7/21/2011	7.3449	0.2596	2.8623	0.7735	835	287	113	1888323
7/26/2011	11.9595	0.6011	6.3581	1.0495	987	300	73	2767119
7/28/2011	15.0708	0.8333	6.2998	1.117	941	313	180	2084243
8/2/2011	5.9809	0.485	5.5477	2.794	2280	673	140	3130076
8/4/2011	11.0746	0.4303	3.3337	2.661	2538	1080	513	3048869
8/11/2011	8.5297	0.3757	4.2924	1.4605	2056	2240	293	5078662
8/16/2011	13.3143	0.5738	3.1642	0.7075	781	1107	227	1644510
8/18/2011	9.4513	0.4098	4.3347	0.845	910	440	207	1726949

8/23/2011	14.4366	0.9221	8.8157	3.0915	13673	1840	1073	2286618
8/25/2011	12.9563	0.9904	7.2373	1.9465	3288	1707	2507	2010339
8/30/2011	8.695	0.5123	5.1398	3.9125	676	500	753	2146852
9/1/2011	6.4488	0.5123	5.2034	1.1955	728	700	293	1800025
9/6/2011	10.649	0.7104	8.5561	1.0685	1872	1087	240	1527776
9/8/2011	4.7138	0.5738	7.8093	0.854	1454	873	213	2235333
9/13/2011	7.4228	0.765	8.2807	1.3595	1073	620	47	1383895
9/15/2011	8.9234	1.2227	11.8294	3.477	500	280	33	1841857
9/20/2011	7.3342	0.5943	9.6049	1.2005	642	540	100	2204885
9/22/2011	6.5629	0.4781	8.5561	0.472	709	440	60	1329823
9/27/2011	7.9885	0.9495	8.2383	0.637	1009	767	100	1320774
9/29/2011	6.8747	0.724	7.0943	0.81	1202	1567	167	2002443
10/4/2011	5.8449	0.6284	6.1886	1.082	395	620	87	1998284
10/6/2011	5.5636	0.7309	5.6801	1.033	357	627	60	1599251
10/11/2011	9.1501	1.0997	8.3125	0.887	361	427	47	1868242
10/13/2011	11.2541	1.0109	10.1292	0.9165	289	633	20	1633532
10/18/2011	5.9249	0.6148	6.1939	0.697	314	407	20	1640071
10/20/2011	10.6459	0.888	8.8898	1.391	295	447	13	1795533
10/27/2011	3.0947	0.2391	3.3814	0.638	493	393	13	1281088
11/1/2011	5.2674	0.403	3.6621	0.574	720	613	7	1766210
11/3/2011	6.7963	0.6148	3.3549	0.455	680	453	0	1701131
11/8/2011	5.9175	0.9358	4.4778	0.289	360	167	0	853707
11/10/2011	6.6191	0.4781	4.0752	0.3915	760	333	7	1356276
11/15/2011	6.2843	0.403	4.0275	0.205	840	113	0	1772679
11/17/2011	3.8502	0.5943	4.0487	0.756	641	267	27	1570599
11/22/2011	6.1468	0.5041	4.9303	0.378	726	233	40	1261261
11/24/2011	4.6541	0.614	4.8292	0.8205	668	107	7	1770328
11/29/2011	7.9029	0.5522	6.7281	0.617	569	60	13	1758271
12/1/2011	8.0479	0.5728	6.1775	1.3425	518	107	0	1855152
12/6/2011	9.2643	0.6552	9.4865	0.525	641	67	0	1436748
12/8/2011	10.7248	0.8887	11.1382	0.4465	841	127	0	1473467
12/15/2011	12.7893	0.7102	12.9416	0.522	781	60	0	1212905
12/20/2011	14.5405	0.8819	11.6888	0.379	381	47	0	1414565
12/22/2011	13.231	0.7102	12.0146	0.307	241	73	0	1078926
12/29/2011	13.0343	0.5934	10.9247	0.548	134	7	0	1176606
1/3/2012	17.3643	0.717	11.3685	0.262	321	60	7	1218324
1/6/2012	26.3826	0.5934	15.8798	0.7045	567	33	20	1489432
1/10/2012	20.3177	0.5179	12.4584	0.505	428	40	0	1193621
1/12/2012	23.4883	0.3599	12.1944	0.4285	428	53	0	1535688
1/17/2012	28.9019	0.6277	16.7112	0.464	1579	187	33	1416834
1/19/2012	23.9822	0.6071	15.1101	0.3495	588	67	13	1789402
1/24/2012	30.504	0.6896	19.5483	0.4635	1883	360	187	2068618
1/26/2012	30.3924	0.7239	18.3404	0.867	782	127	20	1615805
1/31/2012	15.9542	0.5247	8.4079	0.1775	551	127	27	1719703
2/2/2012	16.5266	0.4766	9.0764	0.84	447	60	13	1947454
2/7/2012	13.5934	0.5247	8.5427	0.575	429	47	47	1264580
2/9/2012	16.6816	0.669	9.4472	1.01	335	60	47	1369948
2/14/2012	16.7606	0.8407	11.8742	1.125	393	27	47	1493381
2/16/2012	24.7378	1.1016	16.7506	1.205	454	40	53	2194320
2/21/2012	26.0458	0.7102	15.4022	1.29	808	187	40	2082565

2/23/2012	25.1289	0.6484	13.0146	0.99	934	253	120	1833599
2/28/2012	18.0093	0.5591	11.2056	1.9	810	220	50	1572827
3/1/2012	21.5482	0.8819	14.8011	1.425	821	53	200	2118654
3/6/2012	21.1453	0.5179	10.6213	0.8	1200	413	53	2001169
3/8/2012	19.7423	0.7445	10.7787	1.39	1634	673	40	2116426
3/13/2012	20.3711	0.8613	10.6831	1.445	2829	880	280	2308467
3/15/2012	23.2341	0.8132	12.627	1.095	2890	1533	187	2312165
3/20/2012	22.5977	0.6484	11.009	1.395	5128	2187	593	1416414
3/22/2012	25.9978	0.6552	14.509	1.005	2506	27	2193	1682144
3/27/2012	31.6708	1.3695	17.6607	1.035	4581	1687	747	2236289
3/29/2012	33.7057	1.0124	19.1551	1.27	4137	1787	247	2152725
4/3/2012	29.9017	0.4766	15.4697	1.29	14548	9880	627	2620146
4/5/2012	38.7913	1.0536	21.1944	1.405	6830	4080	247	2324304
4/10/2012	30.0938	0.5797	14.5539	1.7	9197	3100	620	2972026
4/12/2012	38.8411	0.456	18.4191	1.925	3641	613	427	2893660
4/17/2012	38.5255	1.3008	18.3067	3.445	11061	4253	540	3574094
4/19/2012	35.6021	1.4107	16.6831	2.64	4974	2360	327	3149675
4/24/2012	22.8619	0.2706	9.1326	8.355	9898	360	67	2860404
4/26/2012	18.6066	0.8681	7.936	6.375	19140	513	80	2828019
5/3/2012	17.2028	0.0783	3.3124	0.76	6268	307	147	2274857
5/8/2012	16.1156	0.614	2.1438	1.07	6928	467	233	2432220
5/10/2012	11.4689	0.1951	1.891	0.79	2061	147	40	2096261
5/15/2012	11.191	0.0302	1.1326	0.89	1596	147	187	2039191
5/22/2012	15.6748	0.2706	2.2506	1.3	1409	67	67	2526795
5/24/2012	15.2839	0.6896	2.4191	1.125	1588	67	40	3014302
5/29/2012	9.2682	0.6277	1.9022	2.56	3136	607	527	2741626
5/31/2012	11.9505	0.2637	2.2674	3.095	10166	4060	2773	2526023

diatoms	dinoflagellates	date	copepods
9566	2740	4/3/2007	492
6371	5644	4/10/2007	381
13628	6207	4/18/2007	692
25134	21261	4/25/2007	394
6567	19510	5/2/2007	104
16743	10121	5/9/2007	73
40203	10931	5/15/2007	120
18715	10475	5/23/2007	583
4020	16655	5/30/2007	2900
18127	35659	6/5/2007	3200
21263	15436	6/12/2007	1283
82022	19639	6/19/2007	933
1193739	23277	6/26/2007	3550
2705245	36943	7/3/2007	693
3523078	55245	7/10/2007	537
1212669	21458	7/18/2007	822
266731	63880	7/25/2007	4022
125196	62976	8/1/2007	1750
148584	63198	8/8/2007	1203
377056	103237	8/14/2007	628
273288	68726	8/22/2007	386
174552	185957	8/29/2007	1965
328795	137479	9/5/2007	436
71870	53127	9/11/2007	42
196511	74886	9/19/2007	1478
208305	22514	9/25/2007	1592
338860	73702	10/1/2007	1267
109426	34186	10/10/2007	355
352740	87192	10/17/2007	986
486781	724200	10/24/2007	1456
150734	87243	10/31/2007	1478
237627	144125	11/7/2007	628
107529	119696	11/14/2007	1094
149308	48338	11/21/2007	1300
482846	30757	11/28/2007	423
40960	93626	12/5/2007	867
40288	85418	12/12/2007	98
84787	111763	12/19/2007	743
107211	16233	12/28/2007	180
49959	28094	1/2/2008	351
267999	24245	1/9/2008	442
73698	13844	1/16/2008	317
12533	9214	1/23/2008	148
7429	10395	1/30/2008	465
4570	8443	2/6/2008	90
1812	2948	2/13/2008	307
8409	24793	2/20/2008	486
5681	15935	2/27/2008	213
10728	19322	3/5/2008	706

14287	15552	3/12/2008	1422
10909	13518	3/19/2008	705
26189	11898	3/26/2008	2910
14923	10999	4/9/2008	2620
14136	308	4/16/2008	928
288640	13809	4/23/2008	939
543367	25088	5/5/2008	339
263572	10473	5/13/2008	173
1085653	17898	5/19/2008	3550
9548	21474	5/28/2008	2950
22301	7758	6/4/2008	1500
23972	31470	6/11/2008	1050
159685	15906	6/18/2008	3800
102142	7808	6/25/2008	639
201980	9210	6/30/2008	1294
243541	19604	7/2/2008	5072
395470	49225	7/9/2008	2933
371866	46928	7/16/2008	3450
416502	30986	7/23/2008	10075
429047	43553	7/30/2008	3239
565439	49715	8/6/2008	651
130219	98475	8/15/2008	166
422363	71764	8/22/2008	4225
418471	71360	8/27/2008	3417
458423	37830	9/3/2008	1883
394482	107814	9/10/2008	894
154979	33294	9/17/2008	356
120445	39090	9/24/2008	214
136251	79255	10/1/2008	1597
281005	75172	10/8/2008	575
421940	111833	10/15/2008	384
450358	135222	10/22/2008	145
321263	162819	10/29/2008	139
635098	38483	11/4/2008	341
234805	65302	11/7/2008	111
194325	37060	11/10/2008	358
113530	10655	11/19/2008	105
192680	154661	11/26/2008	435
269359	37579	12/3/2008	83
104624	6683	12/10/2008	120
114340	18118	12/17/2008	182
70567	22996	1/14/2009	377
49314	23403	1/21/2009	207
22756	40172	1/28/2009	280
57248	11574	2/4/2009	381
65786	36156	2/11/2009	477
42242	7800	2/18/2009	792
22923	22288	2/25/2009	429
25108	28531	3/4/2009	205
4642	20934	3/11/2009	515

11012	17053	3/18/2009	2339
5346	14470	3/25/2009	1050
38753	23654	4/1/2009	678
9216	11226	4/8/2009	578
3205	4733	4/15/2009	525
10622	14173	4/22/2009	1722
7112	7386	4/29/2009	493
72216	11266	5/6/2009	2538
2812	663	5/13/2009	6010
14104	22297	5/20/2009	4956
27632	44904	5/27/2009	2639
18455	3507	6/5/2009	185
17163	4254	6/10/2009	2213
502714	39198	6/17/2009	1142
656371	41016	6/24/2009	138
292745	53562	7/1/2009	97
50365	88976	7/8/2009	472
32451	142059	7/9/2009	3489
58784	58126	7/15/2009	1739
178570	28301	7/22/2009	1406
104196	78427	7/27/2009	489
29238	53604	8/5/2009	122
1002795	46857	8/12/2009	348
91089	35220	8/19/2009	363
47474	59334	8/24/2009	283
353234	73578	9/2/2009	19
203295	97313	9/16/2009	115
237580	40433	9/23/2009	244
1200203	99982	9/30/2009	79
290184	31096	10/7/2009	155
135187	29755	10/14/2009	4
617492	33174	10/28/2009	69
97947	50933	11/4/2009	55
230195	31237	11/11/2009	194
505485	77591	11/19/2009	428
181565	57340	11/20/2009	232
35289	121046	11/25/2009	546
809202	94298	12/2/2009	445
399307	210650	12/9/2009	544
328102	114488	12/16/2009	629
301351	119335	12/23/2009	439
141524	47083	1/6/2010	367
157582	221398	1/13/2010	76
240002	113855	1/20/2010	428
142917	83075	1/27/2010	216
280028	137785	2/3/2010	97
482535	36592	2/10/2010	91
949549	130507	2/17/2010	72
665014	263184	2/22/2010	684
581134	242614	3/3/2010	292

349294	363285	3/10/2010	143
78411	484556	3/15/2010	288
539530	244822	3/24/2010	115
175486	730152	3/31/2010	393
160456	277118	4/7/2010	12
307835	720975	4/14/2010	511
107815	786163	4/19/2010	1967
81608	198710	4/28/2010	1461
17240	45734	5/5/2010	1606
142665	25948	5/12/2010	117
73495	30895	5/19/2010	6175
62036	17258	5/26/2010	4517
52459	26615	6/2/2010	1372
69323	7289	6/9/2010	355
51464	18425	6/16/2010	77
97764	10168	6/23/2010	3567
50733	4279	6/28/2010	4763
44986	21575	7/7/2010	1561
14170	10658	7/14/2010	7200
27469	15906	7/28/2010	9300
28130	17836	8/2/2010	3056
56919	26414	8/11/2010	2517
18570	12918	8/18/2010	226
26329	7839	8/25/2010	37
33638	21445	9/3/2010	20
43023	19756	9/8/2010	107
24319	18316	9/15/2010	25
124678	15325	9/22/2010	180
53809	13717	9/29/2010	302
185592	13298	10/6/2010	527
84091	13087	10/13/2010	155
24875	13896	10/20/2010	279
16788	17457	10/27/2010	114
23007	10828	11/1/2010	334
878379	16617	11/10/2010	398
59149	9948	11/17/2010	302
92608	16086	11/24/2010	271
26038	22184	12/3/2010	146
10350	19996	12/8/2010	33
20620	35411	12/15/2010	187
7040	16157	12/22/2010	100
21586	27194	12/30/2010	280
21678	35172	1/5/2011	105
34877	18532	1/12/2011	556
11080	27354	1/19/2011	638
6900	29434	1/26/2011	478
34374	15637	2/2/2011	901
8860	8899	2/9/2011	942
34594	34851	2/16/2011	1178
4500	1880	2/23/2011	513

89182	21855	2/28/2011	421
4120	12787	3/2/2011	364
179793	15266	3/9/2011	278
20545	18875	3/16/2011	139
14437	23564	3/23/2011	738
27216	4499	3/30/2011	349
39492	8758	4/6/2011	2950
44510	15385	4/13/2011	538
137803	15724	4/20/2011	706
148754	18764	4/27/2011	2472
94614	46311	5/4/2011	833
382164	50952	5/11/2011	453
324526	38681	5/18/2011	378
86262	55298	5/25/2011	406
1062533	119101	6/1/2011	900
448360	169300	6/8/2011	953
416796	85740	6/15/2011	5900
822739	68480	6/22/2011	1022
293987	61358	6/29/2011	14000
244749	47166	7/6/2011	2122
127354	33705	7/13/2011	5450
16703	22664	7/20/2011	818
25925	34416	7/27/2011	4670
17124	66274	8/1/2011	580
37524	52313	8/2/2011	106
20680	25476	8/3/2011	2706
28443	55657	8/10/2011	2228
35811	30677	8/17/2011	5750
40640	81890	8/24/2011	2272
44711	58190	8/31/2011	755
37875	34375	9/14/2011	2180
4380	18936	9/21/2011	1800
30714	37711	9/28/2011	1306
23690	48147	10/5/2011	470
22731	27936	10/12/2011	1950
94961	98125	10/19/2011	1481
170288	32220	10/26/2011	911
720208	24094	11/2/2011	803
464304	71464	11/9/2011	2397
332396	88937	11/16/2011	1644
171432	65611	11/21/2011	593
152585	48010	11/25/2011	797
138827	61798	11/30/2011	468
58225	19914	12/7/2011	409
36972	15508	12/14/2011	594
494286	35902	12/21/2011	397
1143447	95437	12/28/2011	243
147698	60853	1/4/2012	115
17419	22235	1/11/2012	605
23998	13046	1/18/2012	655

53967	85298	1/25/2012	372
110500	93199	2/1/2012	931
101160	49248		
35899	37532		
19185	41053		
13499	28315		
57005	23785		
72419	27112		
30999	25535		
14749	30053		
36478	33556		
41703	14458		
32776	39310		
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28754	22014		
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20836	10638		
33643	20585		
24219	36880		
13789	12839		
45569	18416		
9579	25894		
7160	34733		
16337	36802		
32973	35432		
67352	40431		
38781	37082		
30194	3220		
18388	26683		
7210	19815		
10829	15116		
6800	9218		
8230	5349		
15909	4150		
10430	5329		
17188	10359		
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16258	6589		
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6120	10298		
8230	13307		
3300	7029		
2390	20596		
18357	4360		
34252	12327		
7140	3060		
9529	13187		
2880	12637		
2590	7509		
17876	9439		

22484	9988
4320	8438
12438	13428
7131	13190
21067	12947
17498	16756
10130	26733
13789	8438
1730	17616
16420	21325
34435	21985
44842	47491
30839	55389
65021	37628
205433	87954
535510	64169
277229	66137
1143447	35784
225424	47999
134579	16847
41631	18485
37737	22085
42278	52907
23999	65066
33580	27991
74662	13046
133287	59445